ADVANCES IN GENETICS

VOLUME II

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Cytoplasmic Inheritance

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CONTENTS

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Glossary of Abbreviations Used

Abbreviation	Interpretation
Epilobium	
l	haploid genome of *E. luteum*
h	haploid genome of *E. hirsutum*
r_1, r_2	haploid genomes of two strains of *E. roseum*
p	haploid genome of *E. parviflorum*
m	haploid genome of *E. montanum*
λ (lambda)	plasmon of *E. luteum*
ἱ (iota with spiritus asper)	plasmon of *E. hirsutum*
πα (pi alpha)	plasmon of *E. parviflorum*
ρ_1, ρ_2 (rho)	plasmons of two strains of *E. roseum*
λ *ll*	*E. luteum*
ἱ *hh*	*E. hirsutum*
λ *lh*	hybrid ♀ *luteum* × ♂ *hirsutum*
ἱ *lh*	hybrid ♀ *hirsutum* × ♂ *luteum*
λ_x *hh*	*luteum* plasmon with *hirsutum* genome, obtained by outcrossing to ♂ *hirsutum* for x generations
Mosses	
Pi	haploid genome of *Physcomitrium piriforme*
Hy	haploid genome of *Funaria hygrometrica*
πι (pi iota)	plasmon of *Physcomitrium piriforme*
‘Υ (ypsilon with spiritus asper)	plasmon of *Funaria hygrometrica*
πι*Pi Hy*	hybrid ♀ Physcomitrium × ♂ Funaria
πι*Pi* Hy^2	hybrid containing one genome of Physcomitrium and two genomes of Funaria in Physcomitrium plasmon

ABBREVIATION	INTERPRETATION
Oenothera	
B, 1	complexes of *Oenothera Berteriana*
v, I	complexes of *Oenothera odorata*
II	complex consisting of four chromosomes from I and two chromosomes from 1, segregating in 1I
βερ (beta, epsilon, rho)	plasmon of *Oe. Berteriana*
όδ (omikron, delta)	plasmon of *Oe. odorata*
Lymantria	
A	gene causing large thoracic spot, obtained from Japanese strains
a	gene causing small thoracic spot, obtained from European strains
ιαπ (iota, alpha, pi)	plasmon from Japanese strains
εὐ (epsilon, ypsilon)	plasmon from European strains
Linum	
M	gene causing pollen fertility derived from procumbens strain
m	gene causing pollen sterility derived from tall strain
π (pi)	plasmon of procumbens strain
τ (tau)	plasmon of tall strain
Paramecium	
K	gene for "killer" action
k	recessive allele causing sensitivity to killer action
κ (kappa)	plasmon necessary for "killer" action

I. Criteria for the Establishment of Cytoplasmic Effects

In sexual propagation, the fusion of a male and a female germ cell forms the starting point for the formation of a new organism. Usually, the same number of chromosomes is contributed by both sexes. It is generally agreed that the genes, carried in the chromosomes, are relatively stable units which are transmitted from generation to generation and are responsible for similarities between parents and offspring. It is not so clear whether extranuclear constituents of the cell also have a certain amount of stability which may be transmitted to succeeding generations. If they do, and if they are able to influence the phenotype of the offspring, a type of transmission different from that of gene transmission will result, which may be called cytoplasmic inheritance.

In the zygote, the largest amount of cytoplasm is contributed by the female germ cell. It should therefore be expected that in cases of cytoplasmic inheritance differences between reciprocal crosses would result.

Usually hybrids from reciprocal crosses are equal, but sometimes differences in reciprocal hybrids are found especially in crosses between different species. However not all of them can be taken as evidence for independent, relatively stable characters of the cytoplasm.

1. *Predetermination and Maternal Inheritance*

There will usually be no difficulty in recognizing reciprocal differences due to different gene distributions, especially in sex-linked inheritance. But difficulties arise when it comes to the distinction between different cases of predetermination and true cytoplasmic inheritance. A number of characters of an organism are already determined in the unfertilized egg, and with respect to characters of this type a hybrid will appear similar to the maternal strain.

This type of determination, which may be called predetermination, is apparently not rare. Most frequently found are cases of predetermination through the genotype of the mother, so-called maternal inheritance. In some cases this is certainly due to transmission of a substance formed under the influence of the genotype of the mother and stored in the egg. In *Ephestia kühniella* the recessive gene *a* determines a reduction of pigment in the eyes, testes and brain of the imago, and of the larval hypodermis and ocelli. In crosses ♀ *aa* × ♂ a^+a, the segregating a^+a and *aa* individuals can be easily distinguished at hatching from the egg by their coloration. But in the reciprocal cross, all offspring show the a^+phenotype, though 50% of them turn out in later development to be *aa*. In this case a substance, now known to be kynurenin, which is necessary for the formation of the pigments, has been formed by the a^+a mother and has been stored in the egg. This is proved by implantation of an a^+a^+ testis into *aa* females, which are then mated to *aa* males. The resulting *aa* progeny have pigmented ocelli and hypodermis, due to kynurenin released by the implanted testis (Caspari, 1936).

Not in all cases of maternal inheritance can transfer of a gene-controlled substance be proved by transplantation experiments. In Drosophila, eggs of females homozygous for the gene "fused" (*fu*) do not develop unless fertilized by a sperm containing a normal X chromosome. Fused eggs from *fu*/+ females are perfectly viable. Clancy and Beadle (1937) have shown that *fu*/*fu* ovaries implanted into wild type females produce eggs which are viable only if fertilized by a wild type, X-carrying sperm. The characteristic behavior of eggs from *fu*/*fu* females is not due to lack of a diffusible substance which is present in wild type and *fu*/+ females, but absent in *fu*/*fu* homozygotes. In cases of this kind, it must remain doubtful whether predetermination has taken place under the influence of a non-diffusible substance provided by the maternal organism and stored

in the egg, or whether the diploid nucleus of the oogonium had determined the cytoplasm of the egg before meiosis.

In other cases, environmental factors are able to influence the germ cells in such a way that the phenotype of the resulting offspring is changed. A number of cases of this type have been reported, for example, in Habrobracon (Kühn, 1927). In this case, a change brought about by temperature in the maternal organism is transmitted to the offspring, presumably by the cytoplasm of the egg.

In all cases of predetermination, there is a tendency of the character to disappear sooner or later in the development of the individual. In many of the cases of maternal inheritance, embryonic characters, such as the color of the chorion in Bombyx, and the endosperm color in Matthiola (Correns, 1900) are those which are affected, and the reciprocally different characters are lost at maturity. In the above quoted case in Ephestia, the increased pigmentation of predetermined *aa* larvae diminishes gradually, but can be demonstrated throughout the whole larval period (Caspari, 1936).

2. *Dauermodifications*

In some cases, environmentally induced cytoplasmic changes have been transmitted maternally through a number of successive generations. These are the so-called dauermodifications which have been described by Jollos (1913, 1914, 1921) and by Sonneborn (1943) in protozoa, by Child (1939), Jollos (1932), Plough and Ives (1935) in animals, and by Hofmann in plants (1927). (See review by Hämmerling, 1929.) In all these cases it is found that an environmental change, *e.g.* temperature or a chemical, induces a phenotypic change which is transmitted purely maternally in animals and plants to their offspring. In all cases, there is a tendency for the cytoplasmic character to become reduced in early generations. For instance, Hofmann (1927) induced by treatment of Phaseolus with chloral hydrate leaf aberrations which were transmitted through the ovum, but not through the pollen, and disappeared in the course of six generations. A bud variation described by Sirks in Phaseolus appeared spontaneously (that is, the inducing factor is not known) and disappeared after eight generations of selfing (Sirks, 1937). This gradual decrease of the effect makes it possible to differentiate between dauermodifications and the relatively stable characteristics of the cytoplasm which, if demonstrated, form the basis for cytoplasmic inheritance.

In mammals and other viviparous animals, there is furthermore the possibility that substances are transmitted from the mother to the embryo by the blood stream, or to the young by the milk. The best investigated case of this kind is a type of mammary tumors encountered in a strain of

mice (Murray and Little, 1935; Bittner, 1936a and b, 1937, 1940, 1941, 1942a and b; Bittner and Little, 1927). In this case, the tendency to form mammary tumors was transmitted wholly maternally in all crosses. It proved possible, however, to induce the same type of tumor in mice from quite different, healthy strains by foster-nursing the young on females from the mammary tumor strain. Further investigations have shown that a tumor inducing substance is formed in the cells of the mice from this strain, and is transmitted to the young through the milk. Once introduced in this way into another organism, the "milk-factor" seems to reproduce itself.

3. *Cytoplasmic Inheritance*

In view of the occurrence of maternal inheritance, dauermodifications and transmission of substances through the egg cytoplasm and through milk, care must be exercised in claiming that a maternal effect is due to a specific quality of the cytoplasm. Reciprocal differences in F_1 are certainly not sufficient, and their unchanged transmission to F_2 may be suggestive, but is certainly not convincing proof. For the same reason, merogony experiments cannot be regarded as evidence for the occurrence of cytoplasmic inheritance, since in most cases the merogonic hybrids cannot be used for breeding. An independent action of the cytoplasm seems, however, demonstrated in Harder's merogony experiments on basidiomycetes (1927). In these organisms, after fertilization, mycelia are formed which contain two haploid nuclei in every cell. The two nuclei in the most apical cell divide simultaneously and in the formation of the new cell wall, three nuclei go into the apical daughter cell, one only remaining in the basal daughter cell. One of the three nuclei of the apical cell is then transferred back to the basal daughter cell by means of an outgrowing tube, the so-called clamp. By destroying the clamp and the apical cell, and severing the basal daughter cell from the remaining mycelium, by means of a micromanipulator, Harder succeeded in obtaining hybrids between two strains of *Pholiota mutabilis* which contained a mixture of the cytoplasms of the two strains and the haploid nucleus of one of the strains. Which one of the strains had given rise to the nucleus could be determined by observing whether it belonged to the + or − sex. It turned out that, in all cases, the nucleus belonged to the + sex. Cells containing a − nucleus in the mixed cytoplasm died. The surviving haploid cells grew out to form mycelia which continued at first to form empty clamps, but gradually lost this ability, indicating gradual loss of a condition necessary for clamp formation. The mycelia continued, however, to show a growth behavior intermediate between that of the + and the − strain, just like the binucleate hybrid. This experiment leads to the conclusion that during mitotic

reproduction a character is transmitted indefinitely for which no nuclear component is responsible and which must therefore be due to the cytoplasm.

Only when a certain character proves to be more or less constant in a series of backcrosses to the paternal strain, or when genic transmission is excluded by substitution of all chromosomes in a species, and dauer-modifications are unlikely since no decrease in the action of the cytoplasm appears in successive generations, evidence for cytoplasmic inheritance seems to be good, unless another interpretation of the facts can be found.

It is well established that one cytoplasmic constituent, the plastids of plants, possesses properties of its own which are transmitted unchanged to the offspring. Their ability to form chlorophyll, and their speed of propagation are certainly under the control of genes, but many crosses are also known, where these characters appear to be dependent on the nature of the plastid itself. Maternal transmission of plastid characters will not be discussed in this review, except for its relation to cytoplasmic inheritance.

II. The Constancy of the Plasmon

About twenty cases of cytoplasmic effects have been described in the literature. Some of them have been carried only up to F_2, or have not been published extensively. All of them will be found in the References at the end of this paper, but it is proposed to treat extensively only those cases in which cytoplasmic transmission has been established through repeated backcrosses or through chromosome substitutions. Other cases will be mentioned wherever they contribute to our understanding of the processes involved.

1. *Gynodioecy in Cirsium*

A typical case has been described by Correns (1908, 1916, 1928). In *Satureia hortensis* and *Cirsium oleraceum*, both monoecious and female plants exist. Monoecious plants always give monoecious offspring, while female plants which must be pollinated by pollen from monoecious plants give female progeny only. In order to exclude the possibility of selective gone mortality, as has been proposed by East (1934), Correns crossed ♀ *Cirsium oleraceum* to the related *Cirsium canum* in which female plants do not exist, and which is therefore assumed not to possess genes for "femaleness." After six generations of backcrosses to *C. canum*, in which the majority of the genes must have been replaced by *canum* genes, the offspring was still exclusively female. It is concluded that the cytoplasm of female *C. oleraceum* plants possesses some properties which permit only the female sex organs to develop. These properties have proved to be unchanged through six generations under the influence of foreign genes.

Stable properties of the cytoplasm which may be transmitted to the

offspring will be called "plasmon" in the ensuing discussion. This term is not supposed to imply any notions about the nature of these cytoplasmic properties, and is especially not intended to suggest an independent cytoplasmic agent of a function similar to the gene. It is only used to designate properties which are relatively independent of genic and environmental influences. According to a usage first introduced by von Wettstein, and followed by a number of workers in the field, notably Sonneborn, the plasmon will be designated by appropriate Greek letters, the genome being designated by letters of the Latin alphabet. The gene and plasmon symbols used in this paper are collected in the glossary. Michaelis has objected to the use of Greek letters which has proved cumbersome in his complicated crosses. But it seems to be convenient for the purpose of the present review.

2. *Pollen Sterility in Maize*

A case which in many respects is similar to Correns' Cirsium case has been described by Rhoades (1933) in maize. He obtained a male sterile line which when outcrossed to pollen from any normal line gave more or less exclusively male sterile offspring. No evidence for segregation of male sterility was found in these cases. Furthermore, substitutions of marked chromosomes from male fertile strains for the corresponding chromosomes of the male sterile line did not result in improved pollen fertility, even though every one of the ten chromosomes of maize was tested. It must be concluded that no chromosomal male sterility factors are present. A further possibility for tests arises from the fact, that male sterile plants sometimes produce a certain amount of pollen. Forty-nine of these pollen producing plants from the male sterile line were progeny tested by pollination with pollen from male fertile strains. Forty-six of them gave varying percentages of male sterile offspring, while three gave normal offspring only. It must be concluded that most of these *phenotypically* normal plants *actually* carried the necessary qualities for male sterility. The existence of "male sterile" plants which produce viable pollen permitted the observation that the factors responsible for male sterility are not transmitted by the pollen. Rhoades rejects the possibility that in the male sterile line only gones carrying a dominant gene for male sterility are fertilized. This hypothesis would lead to the expectation that abnormal segregation ratios would be found in backcrosses to strains carrying marker genes on the chromosome homologous with the male sterility chromosome. This was not the case. A cytoplasmic condition transmitted independently of the genes must therefore be responsible for this case of male sterility.

3. *Pathogenicity in Rusts*

Other cases of direct cytoplasmic transmission of a character have been described by Johnson, Newton and Brown (1934, 1940; Newton and Johnson, 1932) in *Puccinia graminis* Tritici and Avenae. In these rusts, the sexual stages are represented by the uninucleate pycniospores which are formed on the alternate host. + and − sexes exist. In crossing, exudate from single pycnia is transferred to another infected leaf. If the pycnia are of different sexes, they will copulate and cause the formation of binucleate aeciospores, the infective stage for the cereal. Different physiological races were crossed, which were distinguished by a particular degree of pathogenicity towards particular inbred strains of the host cereal. While in many cases clear-cut Mendelian segregations were observed, two cases could be found in which reciprocal differences existed, the hybrid showing the same pathogenic character towards a certain host as its parent to which the pycniospores had been transferred. Since the transferred pycniospores are minute in size, carrying a nucleus but only small amounts of cytoplasm, it is concluded that the particular pathogenicity is transmitted by the cytoplasm of the "maternal" strain. This "matrocliny" could be transmitted unchanged through three generations of selfing. Similar cytoplasmic effects have been observed by d'Oliveira (1939) in *Puccinia anomala.*

4. *Inhibitions in Epilobium*

More complicated is the situation in Epilobium crosses which have been extensively investigated by Lehmann and his collaborators and by Michaelis and his collaborators. Michaelis (1933) made reciprocal crosses between *Epilobium hirsutum* and *E. luteum*. The F_1 ♀ *hirsutum* × ♂ *luteum* is completely pollen sterile; the reduction division appears cytologically normal, but later on, first the tapetum and then the microspores degenerate. If these hybrids are backcrossed to *luteum* pollen, the disturbances are greater than in the F_1 hybrid, the degeneration of the tapetum and pollen starting earlier and progressing faster. Furthermore, vegetative disturbances appear. The next backcross generation does not develop beyond the seedling stage. The increase in inhibiting activity of the *hirsutum* plasmon in successive generations excludes the possibility of describing this effect as due to a dauermodification, since the gradual decrease of the effect is regarded as a peculiar feature of dauermodifications.

In the reciprocal hybrid, ♀ *luteum* × ♂ *hirsutum*, no plant was completely pollen sterile. The pollen fertility as judged by the microscopic appearance of the pollen was reduced as compared with the parent plants. In later generations of backcrossing to ♂ *hirsutum*, an increasing number of pollen sterile plants appeared which, from the fifth generation on, made

up about 40% to 70% of all progeny. Of the remainder some are fully fertile, like the parents, while others are partially pollen fertile like the F_1 hybrid. Shoots of pollen sterile plants sometimes become fertile; these belong always to either one of the two fertility groups. The death of the pollen grains takes place in all cases after a cytologically normal meiosis.

While the first generations of backcrosses show typical segregation for morphologic characters, the plants are, from the eighth generation on, nearly identical with *hirsutum.* In some physiological characters there is still some similarity to *luteum*, such as in the independence of pollen sterility on weather conditions, higher resistance against bad nutrition and against infection with mildew, size of the pollen grains and reaction to long and short day and similar climatic factors (Fürtauer, 1941; Michaelis, 1935b and c, 1939). But in general, it appears similar to *hirsutum* and must therefore be assumed to carry mostly, though probably not exclusively, *hirsutum* genes. Plants of this type will therefore be designated as λ *hh*, as opposed to ι *hh*, the original *E. hirsutum.*

Eight generations of backcrossing appear to be a short time for the replacing of the complete maternal set of genes. But two factors seem to have contributed to the speed of replacement, conscious selection by Michaelis for paternal characters in the first generations of backcrossing, and inviability of gametes with unbalanced genomes. The latter is suggested by the finding that in pollen sterility, tetrads with 0, 2 and 4 inviable pollen are more frequent, than tetrads with 1 or 3 inviable pollen (Michaelis, 1933).

The characters resembling *luteum* are supposed to be dependent on the cytoplasm. The argument is based on individual reciprocal crosses between λ *hh* and ι *hh,* in which in each case the same two plants were used both as mother and as father. Therefore, even if some *luteum* genes should still be present in heterozygous condition in the λ *hh* plants, they would be expected to be identical in the crosses in both directions. Plants from the cross ♀ ι *hh* × ♂ λ *hh* were all fertile, like typical *hirsutum;* plants from the reciprocal cross consisted of pollen sterile and pollen fertile plants, and of plants with reduced pollen fertility, like the original λ *hh.* It is concluded, that the pollen sterility and reduced pollen fertility characteristic of λ *hh* are transmitted maternally, and therefore through the cytoplasm. The higher resistance of the pollen fertility of λ *hh* against bad weather has been shown to be inherited in the same way.

The main argument for an independent action of the plasmon has been derived from reciprocal outcrosses of λ *hh* plants to *E. luteum* (λ *ll*), which will place the hybrid genome *hl* in both directions into plasmon λ (Table 1).

After seven generations of outcrossing of the hybrid to *hirsutum*

pollen, all offspring of ♀ λ*hh*×♂ λ*ll* were fertile, just like the original λ *hl* from ♀ λ*ll*×♂ ι*hh*, and are also in all other respects identical with it. In other words, the hybrid genom constitution *hl* behaves, when the mother has been λ *hh*, the same way, as though the mother had been *luteum* and not *hirsutum*. This result indicates, that it is the *luteum* cytoplasm which is responsible for the reciprocal differences in F_1 *luteum*× *hirsutum* (Michaelis, 1933). Since the outcrosses to ♂ *hirsutum* have been continued through fourteen generations, it appears that the *luteum* plasmon has retained its specific action through this time.

TABLE 1

Pollen Fertility in Crosses between *Epilobium hirsutum* and *E. luteum* (after Michaelis, 1933)

Cross	Formula	F_1 Formula	F_1 Pollen Fertility
♀ *luteum* × ♂ *hirsutum* . . .	♀ λ*ll* × ♂ ι*hh*	λ*lh*	All plants fertile
♀ *hirsutum* × ♂ *luteum*	♀ ι*hh* × ♂ λ*ll*	ι*lh*	All sterile
(♀ *hirsutum* × ♂ *luteum*)$_s$ . .	♀ ι*lh* × ♂ λ*ll* . . .		Lethal
(♀ *luteum* × ♂ *hirsutum*)$_n$ . .	♀ λ*lh* × ♂ ι*hh* . . .	λ*hh*	Viable; either 100% fertile, or 50% fertile, or sterile
♀ *hirsutum* with *luteum* plasmon × ♂ *luteum*	♀ λ*hh* × ♂ λ*ll*	λ*lh*	All plants fertile
♀ *luteum* × ♂ *hirsutum* with *luteum* plasmon	♀ λ*ll* × ♂ λ*hh*	λ*lh*	All plants fertile
♀ *hirsutum* with *luteum* plasmon × ♂ *hirsutum*	♀ λ*hh* × ♂ ι*hh*	λ*hh*	Either 100% fertile or 50% fertile or sterile
♀ *hirsutum* × ♂ *hirsutum* with *luteum* plasmon	♀ ι*hh* × ♂ λ*hh*	ι*hh*	Fertile

It has not remained unchanged, though. This is indicated by the fact that, in the same year under identical environmental conditions, λ8*hl* (the number indicates the number of generations of backcrossing) from a cross ♀ λ7*hh* × ♂ λ*ll* had on the average $20.49 \pm 0.29\%$ fertile pollen, while the individual reciprocal λ0*hl* had $28.99 \pm 0.62\%$ fertile pollen (Michaelis, 1933, 1938). This means that during the seven generations of outcrossing to *hirsutum*, λ has become changed in such a way as to inhibit pollen fertility more strongly. Since the genotype *hl* is completely pollen sterile in ι, this fact is regarded as a gradual approximation of λ towards ι under the influence of the *hirsutum* genome. Differences in the

pollen sterility of *hl* and *hirsutum* hybrids with other Epilobium species in λ5, λ8 and λ11 indicate, that this change in the constitution of λ under the influence of *hirsutum* genes is gradual (Michaelis and Wertz, 1935). Similar differences between λ0 and λ10 have been found for petal size (Michaelis, 1938).

Michaelis tries to exclude the possibility that the gradual change of λ is brought about by admixture of small amounts of ι transmitted through the *hirsutum* pollen. His argument is based on his interpretation of the occurrence of fertile sectors on pollen sterile λ*hh* plants. They are regarded as due to small amounts of ι cytoplasm transmitted through the pollen which become predominant in certain cells giving rise to a fertile shoot, since the constitution ι*hh* (original *hirsutum*) is pollen fertile. This argument is based on an analogy with the occurrence of pure green and pure white shoots on variegated albomaculatus plants, and on the fact that sterile plants having sectors with fertile flowers appear with about the same frequency as that with which white plastids were transmitted through the pollen from a paralbomaculatus Epilobium (about 0.22%, Michaelis, 1935a, 1938). On the other hand, since ι cytoplasm causes the *hl* hybrid to be completely sterile, *hl* plants derived from fertile sectors should be less fertile than those derived from sterile parts, under Michaelis' hypothesis for fertile sectors. This has not been found, and this fact forms the main argument for the theory that the gradual change of λ in λ*hh* is due to the *hirsutum* genome. The change which never succeeds in approximating the effects of ι is likened to a dauermodification.

It must be concluded, that the plasmons of *E. hirsutum* and *luteum* are certainly different, and that this difference has been maintained for fourteen successive generations of backcrossing. However, during this time a change of the *luteum* plasmon has taken place which changes its action in the direction of the *hirsutum* plasmon. It should also be emphasized, that the differences between the two plasmons are slight and not obvious when they work with a more or less homozygous *hirsutum* genome, and are most obvious in the F_1 *hl* hybrid. The differences in *luteum* plasmon of different generations of outcrossing have been confirmed in crosses of λ*hh* to *Epilobium roseum* and *E. montanum* (Michaelis and Wertz, 1935).

In another series of experiments, cytoplasmic differences between different geographic strains of the same species *Epilobium hirsutum* have been studied. Lehmann and his students crossed different strains of *E. hirsutum* to other species, especially *E. adenocaulon* and *E. parviflorum* (Lehmann, 1936, 1939b and c; Lehmann and Schnitzler, 1932). When *hirsutum* was the mother, he obtained inhibitions of different degrees, the degree depending on the particular *hirsutum* strains used. Hybrids between the different *hirsutum* strains did not show any abnormalities (Lehmann,

1941). But when they were outcrossed to *adenocaulon*, they gave segregation into inhibited and uninhibited plants. These segregations will be discussed below. The specificity of the plasmon of the different *hirsutum* strains is shown by the fact that the inhibited plants show a stronger degree of inhibition, if a strongly inhibiting strain had been used as the mother of the original *hirsutum* hybrid, than if a weakly inhibiting strain of *hirsutum* had been used as the mother. In these crosses, the plasmon differences between different *hirsutum* strains show up only in the presence of a species hybrid genome. By using a large number of *hirsutum* strains, especially by Lehmann and Schnitzler (1932), a continuous series of inhibiting plasmons could be established.

These results have been amply confirmed by Renner and Kupper (1921), Brücher (1938, 1940b, 1943) and Michaelis (1940b, c and d, 1942). Michaelis (1940b), working with 39 *hirsutum* strains of different origin, found one strain, Jena, which when outcrossed to different *hirsutum* strains gave observable reciprocal differences in F_1. If the other strain was used as mother, the hybrids were more or less normal, while in Jena cytoplasm inhibitions could be observed. A series of inhibitions could be established, ranging from reciprocally equal crosses over slight inhibitions, in Jena plasmon (expressed by dense mottling of the rosette leaves, differences in color and size of the leaves, heterosis in vegetative growth and reduction in petal size) and over definitely inhibited stunted hybrids (with variegated leaves of irregular shape, decreased anthocyan content and increased chlorophyll content, with small flowers and high degree of pollen sterility) to hardly viable dwarf plants. Hybrids with two South African strains are inviable in Jena cytoplasm.

The degree of inhibition is dependent mainly on the strain which has been used as the father, but also on environmental factors. For example, the reciprocal differences of the Jena × Munich hybrids disappear at low temperature (Michaelis, 1940d), while the ♀ Jena × ♂ Parys (South Africa) hybrid dies at high temperature as an embryo of a few cells, but may come to develop cotyledons under the influence of low temperature (Michaelis, 1942a). It is important that all these variations change only the place of the plant in the inhibition series, *i.e.*, the different characters influenced by the Jena plasmon vary concomitantly. The only exception to this rule is pollen sterility, expecially in the weakly inhibited hybrids.

The same inhibitory series can be observed in morphological characters, for example, petal size, of the *hl* hybrid in different cytoplasms. In this case, the cytoplasms of different *hirsutum* strains cause inhibitions according to their inhibitory action on intra-*hirsutum* hybrids (Michaelis, 1937, 1938) (Table 2).

The constancy of the plasmon differences between different hirsutum

strains has been shown by Michaelis by repeated backcrossing of one strain as mother to other strains and finally outcrossing to *luteum* (1940c). For example, the hybrid ♀ Jena × ♂ Munich was outcrossed as female for three generations to strain Munich. The resulting plants should

TABLE 2

Relative Petal Size of *hl* Hybrids in Different Plasmons (*luteum* Plasmon = 100%), after Michaelis, 1937

PLASMON OF STRAIN..	*luteum*	Munich	Stuttgart	I	Hohenheim	Jena
PETAL SIZE IN %....	100	85	73	57	48	2

contain a majority of Munich genes in Jena cytoplasm. The third generation, outcrossed to *luteum*, gave *hl* hybrids showing the inhibitions characteristic for Jena plasmon. Exactly the same result has been obtained by Brücher (1938, 1940b) who crossed his *hirsutum* strains back for four generations. The cytoplasmic differences between different *hirsutum* strains have therefore been kept constant through three or more generations in the presence of different genomes.

5. *Sterility in Streptocarpus*

A case which in some aspects resembles Correns' gynodioecy in Cirsium has been investigated by Oehlkers (1938, 1940). The genus Streptocarpus, an African flowering plant, is always monoecious. Of the five stamens the flower contains, two only are fertile, while the others are empty staminodia. In crosses between the species *S. Wendlandii* and *S. Rexii*, reciprocal differences are found. The F_1♀ *Rexii* × ♂ *Wendlandii* is morphologically normal; but progeny tests show that a large percentage of the ovules are sterile. In the reciprocal cross, the ovules are fertile, but the two fertile stamens are transformed into sterile staminodia, *i.e.* the plants are male sterile. In F_2, segregation into male fertile plants and plants of different degrees of male sterility was found. If the F_1 hybrid (♀ *Wendlandii* × ♂ *Rexii*) was backcrossed to *Rexii* pollen, a segregation in the ratio 1:1 into male sterile and fertile progeny was observed. Different degrees of sterility were found, the highest one being a transformation of all five stamens into well-developed carpels, with pistil and ovules. In other words, the male sex organs have been completely transformed into female ones. This effect has been maintained without decrease through five generations of backcrossing to *Rexii* pollen. It must be concluded, that the cytoplasm of *Wendlandii* acts in the direction of female development, while *Rexii* plasmon seems to inhibit it.

6. *Matrocliny in Mosses*

Crosses between different species of mosses have been extensively studied by von Wettstein (1924, 1926, 1928) and his students. Differences between strains of the species *Funaria hygrometrica* segregated in a typical Mendelian fashion. But in hybrids between the species *F. hygrometrica* and *F. mediterranea,* reciprocal differences were obtained; the hybrid sporophytes resembled the maternal species in shape, size and color and peristome formation. The hybrid gametophyte could be observed in regeneration experiments. If part of the diploid sporophyte is forced to regenerate, it forms a protonema which grows into a gametophyte which is diploid instead of haploid. Hybrid gametophytes were obtained in this way, and showed matrocliny in the shape of their leaves, the length of the mid-vein of the leaves, and in the shape of the paraphyses.

The haploid spores produced by the diploid hybrids were also grown to gametophytes. In this experiment, different results were obtained with the different characters. In the shape of the paraphyses, different types of offspring varying from the purely maternal to the purely paternal type, were found. The mid-rib of the leaves, on the other hand, showed always the pure maternal phenotype. With respect to leaf shape, large variability was encountered; but in both reciprocal crosses those classes resembling the mothers were most frequent, while the purely paternal classes were absent. Since gamete sterility and seedling mortality were very low, this result cannot be explained by preferential mortality, and leads to the conclusion, that the maternal cytoplasm influences the shape of the leaf and the formation of its vein. This conclusion was borne out by crossing the most paternal plants to the pure paternal species as female parent. The progeny of this cross was identical with the pure species and did not segregate in later generations.

In the intergeneric cross ♀ *Physcomitrium piriforme* × ♂ *Funaria hygrometrica*, the great majority of the F_1 haplonts have mostly maternal characters. Since in this case the spore mortality was high, it was concluded that many genes of *Funaria hygrometrica* are not viable in $\pi\iota$ cytoplasm. For if unbalanced genomes were the only reason for inviable gones, wholly paternal plants should be found occasionally, which is not the case. The reciprocal cross could be obtained only exceptionally. But its haploid offspring also showed mostly *F. hygrometrica* characters.

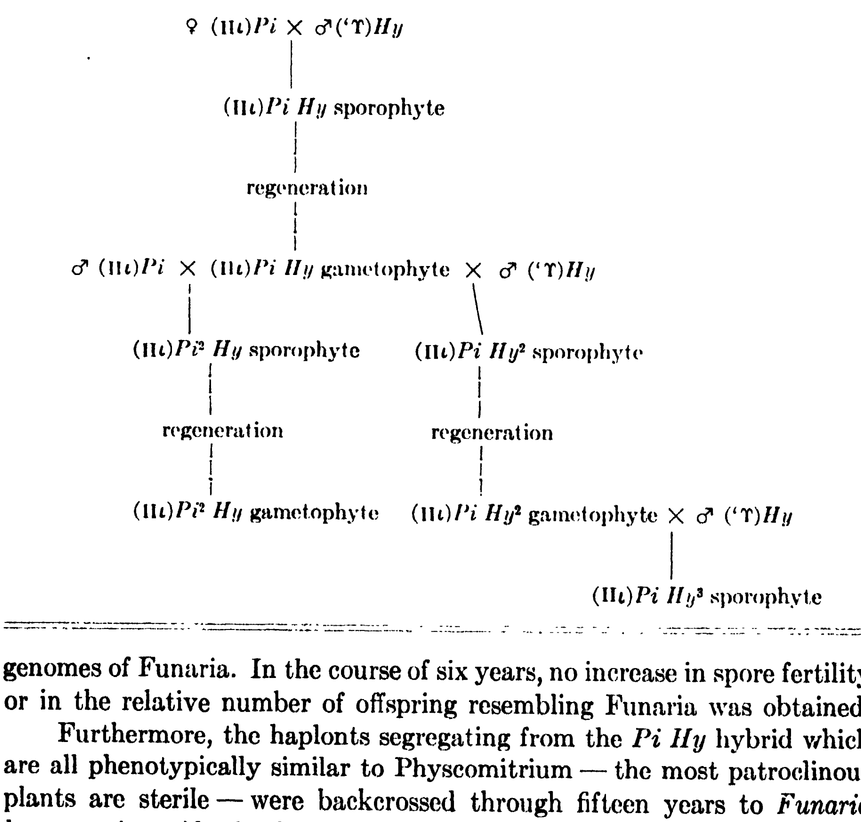
In the following discussion, $\pi\iota$ will designate the plasmon of *Ph. piriforme*, *Pi* and *Hy* one set of genes of the respective species. A number behind the genome indicates the number of genomes of the particular kind present.

The constancy of the $\pi\iota$ plasmon under the influence of *Hy* genes has been shown by von Wettstein in two different ways (1930). (See Table 3).

By regeneration of the *Pi Hy* hybrid, the diploid hybrid gametophyte can be obtained and backcrossed repeatedly to *F. hygrometrica*. In this way, the triploid *Pi* Hy^2 and tetraploid *Pi* Hy^3 were obtained, in which the Physcomitrium plasmon is exposed to the action of two and three full

TABLE 3

Scheme for Obtaining Different Genomatic Constitutions in Mosses



genomes of Funaria. In the course of six years, no increase in spore fertility or in the relative number of offspring resembling Funaria was obtained.

Furthermore, the haplonts segregating from the *Pi Hy* hybrid which are all phenotypically similar to Physcomitrium — the most patroclinous plants are sterile — were backcrossed through fifteen years to *Funaria hygrometrica*. Also in this experiment, no change in phenotype or fertility of the *Pi Hy* hybrids was observed. The constancy of the plasmon of *Physcomitrium piriforme* seems to be unchanged by the influence of *Funaria hygrometrica* genes.

7. *Reciprocal Differences in Oenothera*

The genus Oenothera has shown advantages for the study of cytoplasmic inheritance. Most species of this genus are complex heterozygotes. By means of repeated translocations, a chromosome configuration has

arisen, in which whole chromosomes do not pair at meiosis but the two ends of each chromosome pair with ends of different chromosomes. All chromosomes are therefore arranged in a ring at diakinesis and alternate chromosomes are pulled to different poles at reduction. The genetic effect of this mechanism is that the gametes carry either one of two complexes which exist in the species. The species is kept constant by the facts that complex homozygotes are inviable, or that gametes carrying one particular complex are lethal in one sex. Crossing over is reduced or absent in the original species, but is found frequently in hybrids between different species. Reciprocal differences have been found by Rudloff (1929) in hybrids of *Oe. purpurata* and *Hookeri*, and Schwemmle and his students in crosses involving *Oe. Berteriana*, *Oe. odorata*, *Oe. brachycephala* (Hausteir, 1939), *Oe. argentinea* (Schwemmle and Zintl, 1939) and some related species. The crosses between *Oe. Berteriana* and *Oe. odorata* have been the most extensive ones (Schwemmle, 1932, 1941; Schwemmle *et al*, 1938).

Oenothera Berteriana and *Oe. odorata* are typical isogamous complex heterozygotes. The complexes of *Berteriana* are designated as B and 1, those of *odorata* as v and I. Therefore, in crosses between the two species, four genotypes should be expected, BI, Bv, 1I and 1v. Actually, only three genotypes were found in both reciprocal crosses. In the cross ♀ *Berteriana* × ♂ *odorata*, the constitution Bv is missing, probably because the complex v is only occasionally able to fertilize B ovules. In the reciprocal cross ♀ *odorata* × ♂ *Berteriana*, the Bv is found, and is well developed. The BI and 1v are present, but weak, yellowish in color, and frequently infertile. The 1I are lethal and can be found in the abortive seeds. Their development has been studied by Binder (Schwemmle *et al*, 1938).

The different types obtained in this way have been continued by selfing through 11 to 14 generations. Genetically, they have bred true, without any indication of segregation, except for a few newly appearing types: $\beta\epsilon\rho$BI, $\beta\epsilon\rho$1v and $\beta\epsilon\rho$1I gave rise to two types of plants which could be identified as II and vv in *Berteriana* plasmon. Furthermore, in $\beta\epsilon\rho$1I, a new complex II is formed consisting of four chromosomes from 1 and two from I. The seventh chromosome in crosses involving 1, I and II, which is characterized by a dominant gene *T* for a purple dot on the petal, and a recessive pollen sterility factor in the homologue derived from 1, is distributed at random.

Generally speaking, there seems to be a fairly typical complex heterozygote situation, with the exception, that the combinations II, vv and 1I are viable in *Berteriana* cytoplasm or rather, as will be shown in a later paragraph, with *Berteriana* plastids, but not with *odorata* plastids. Furthermore in BI and 1v *odorata* plastids are yellowish and the plants corre-

spondingly weak. The reason, as will be discussed later, is that *odorata* plastids are more sensitive to certain genotypes than *Berteriana* plastids. The *odorata* plastids in BI and lv are very large and pale and the number per cell is reduced, indicating an inhibition of the development and propagation of the plastids (Sturm, reported in Schwemmle *et al*, 1938).

It should also be mentioned, that from crosses of the type ♀ βερlv × ♂ βερBI the combination βερvI, *i.e.* the genome of *odorata* in *Berteriana* cytoplasm and plastids could be obtained. The opposite ὀδBl could not be obtained, and seems to be lethal.

The first reciprocal difference is therefore, that II, vv, Bl and lI are lethal, and BI and lv weak in *odorata*, but not in *Berteriana* cytoplasm. To this are added a number of other differences, of which the following have been studied: pollen size, the shape of the leaves, the size of the petals, the length of the "hypanthia" or "corollary tubes," *i.e.* the tubes formed by fusion of the lower part of the petals and the calyces.

Oenothera odorata has longer hypanthia and larger petals than *Oe. Berteriana*. Its leaves are rather lanceolate and have a smooth margin, while those of *Berteriana* are broader with a dentate margin. The size of the pollen varies around 140 μ in *Berteriana* and around 160 μ in *odorata*.

In the crosses, the hypanthia are always shorter in *Berteriana* cytoplasm than in *odorata* cytoplasm, independently of which gene complex is compared. In petal size, the situation is more complex, since lv, Bv and vI have larger petals in *odorata* cytoplasm, than in *Berteriana* cytoplasm, while in ὀδBI the petals are smaller than in βερBI. The leaves are matroclinous in shape both in BI and lv, those of the ὀδBI being almost indistinguishable from *odorata* leaves. With respect to pollen size, haploid segregation is found. BI and lv give bimodal distribution curves for pollen size, and it is assumed that the larger mode corresponds to the I or v pollen, the smaller mode to the B or l pollen. This is borne out by the fact, that from combinations in which only one type of pollen is expected, such as II, vII, BII (the complex II is never transmitted by pollen) unimodal curves are obtained. The reciprocal differences can be best observed in lv. In βερlv, the l pollen is equal in size to that of Berteriana, while the v pollen is somewhat smaller than *odorata* pollen, *i.e.* somewhat matroclinous. In ὀδlv, both types of pollen grains are larger than in the reciprocal cross, the v pollen being equal in size to *odorata* pollen, while the l pollen is somewhat larger than that of *Berteriana*.

In successive generations of selfing important changes go on. The weak lv and BI in *odorata* cytoplasm gradually recover. Their plastids become small and green after four or five generations of selfing, and are indistinguishable from normal plastids. Concomitantly, the plants become larger in size, healthier, and completely fertile.

During this process of recovery, the leaves of ὀδlv also change, so that their matrocliny disappears. In ὀδBI, however, the maternal influence does not diminish. The corollary tubes of all combinations become shorter in successive generations, except for ὀδlv, in which they become longer. The reciprocal differences which cause the corollary tubes to be shorter in *Berteriana* than in *odorata* cytoplasm are maintained through all 11 to 14 generations of selfing, since the reduction in hypanthium length is of a similar size in reciprocal constitutions. Correspondingly, the petals become smaller in successive generations of selfing in most complexes, except for ὀδlv, where they become larger, and for βερvI, in which no change in petal size could be observed. Also with respect to the pollen size, changes are found in successive generations. In *Berteriana* cytoplasm, B and l pollen grains remain constant, while the pollen containing complexes from odorata, v and I, show a tendency to become gradually smaller. In *odorata* cytoplasm on the other hand, not only v pollen, but also pollen carrying the complex l derived from *Berteriana* are constant. B pollen probably becomes larger in *odorata* cytoplasm, but the evidence is not conclusive in this case.

The analysis of the changes going on in successive generations has led to interesting and unexpected results which have a bearing on the question of collaboration between genes, cytoplasm and plastids. These results will be discussed in later parts of this review. For the present, it should be emphasized, that in spite of gradual changes in successive generations the same complex heterozygotes show differences in *Berteriana* and *odorata* cytoplasm after 11 to 14 generations of selfing. These differences concern hypanthium length, petal size, pollen size and the shape of the leaves in BI.

8. *Cytoplasmic Effects in Drosophila and Habrobracon*

All the cases described up to now concern plants. But there are a number of cases known in animals. Two will be described here, body color in adult Habrobracon, and CO_2 sensitivity in Drosophila.

Kühn (1927) crossed two laboratory strains of Habrobracon which had been obtained by selection from wild material. One of the strains was considerably darker than the other. The F_1 females were intermediate, but those from light mothers were significantly lighter than the females obtained from the reciprocal mating. This difference between reciprocal matings was maintained both in the haploid sons produced parthenogenetically by the hybrid females, and in their daughters produced in backcrosses to males of the light strain. The sons produced by females from this backcross showed the same significant difference between the reciprocals. The plasmon of the light strain has therefore the tendency

to cause animals to be lighter than animals with the same gene combination in cytoplasm of the dark strain, and this feature has been transmitted unchanged for the three generations investigated.

In Drosophila, L'Héritier and Teissier (1937, 1938a and b) have described a CO_2 sensitive strain which breeds true for this character. CO_2 sensitive and resistant animals can be easily distinguished, and there is no intergradation. The F_1 from a cross ♀ sensitive × ♂ resistant is always sensitive, and behaves in further backcrosses to resistant males like the sensitive strain. The cross ♀ resistant × ♂ sensitive gives always both resistant and sensitive offspring in varying proportions. The resistant F_1 animals do not transmit sensitivity to their offspring, nor do the sensitive F_1 males. Some of the F_1 females have also only resistant offspring, but most of them produce resistant and sensitive progeny in various proportions.

The independence of this CO_2 sensitivity of the chromosomes has been proved by outcrosses of the sensitive strain to males in which all three autosomes were marked by the dominant genes *Cy*, *H* and ey^D. The resulting sensitive $Cy/+\ H/+\ ey^D/+$ males were outcrossed to resistant females. The offspring were partly sensitive and partly resistant, independently of the segregation classes. Since in Drosophila crossing over does not occur in the male, $Cy/+\ H/+\ ey^D/+$ males from this cross do not possess any chromosome from their sensitive grandmother. The fact that these males are sensitive excludes transmission of this character by the chromosomes. By crossing the above mentioned $Cy/+\ H/+\ ey^D/+$ sensitive males to resistant attached-X females, sensitive females of the constitution $\widehat{XX}Y\ Cy/+\ H/+\ ey^D/+$ which did not contain any chromosome from the original sensitive strain have been obtained. If they were crossed to their brothers, they transmitted their sensitivity to their offspring, maintaining the character through four generations.

In this case, a character has been transmitted extra-chromosomally through a number of generations. The only difference to the cases considered above is the possibility of transmission through the sperm as indicated by the CO_2 sensitivity of part of the offspring from a cross ♀ resistant × ♂ sensitive.

The cases described above seem to demonstrate, that transmission of certain characters outside the chromosomes occurs. Without making any assumptions as to the nature of this transmission, it must be assumed to be located in the cytoplasm. These cytoplasmic characteristics show a great variation with regard to their constancy in crosses to other strains. In some cases, such as a maternally transmitted character in Nicotiana described by Honing (1930, 1932), the peculiarity of the cytoplasm seems to be lost in a few generations of outcrossing. Honing crossed different species and strains in Nicotiana which were distinguished by the presence

or absence of the need for light for germination to proceed. In F_1, 81.3% of the seeds germinated in the dark, if the indifferent plant had been the mother, while in the reciprocal cross only 16.4% of the seeds could germinate in the dark. This reciprocal difference was maintained in F_2, but the difference between reciprocals in F_2 was only about one-fourth of the difference found in F_1. In *λhh* Epilobium (Michaelis, 1933), a slight change in the direction of the paternal character appears after a number of outcrosses, while in the moss (von Wettstein, 1930) and Oenothera (Schwemmle *et al*, 1938) cases no change in plasmon could be observed in several generations. It may therefore be concluded, that a plasmon, *i.e.* certain transmissible characteristics of the cytoplasm, exists which is more or less stable under the influence of foreign genes and can be reproduced through several generations independently of the genes present in the organism.

III. The Phenotypic Action of the Plasmon

It appears, from the description of cases in which cytoplasmatic inheritance seems to be involved, that the phenotypic expression of cytoplasmatic differences is varied. It appears possible, however, to find a common denominator if all different types of expression are studied.

1. *Purely Maternal Transmission of Characters*

Cases in which a certain character is transmitted purely maternally are rare. Some of the cases mentioned above, gynodioecy in Cirsium (Correns, 1928), male sterility in maize (Rhoades, 1938) and CO_2 sensitivity in Drosophila (L'Héritier and Teissier, 1937, 1938a and b) seem to be completely dependent on the cytoplasm. The same is true for some of the characters studied in mosses by v. Wettstein and his students, especially the formation of the mid-rib of the leaf in *F. hygrometrica* × *mediterranea* crosses (von Wettstein, 1926, 1928) and the protonema regeneration in ♀ *Ph. piriforme* × ♂ *F. hygrometrica* hybrids (Schwanitz, 1932).

2. *Matrocliny*

Cases of purely maternal transmission of a character, however, are the exception rather than the rule. More frequent is a certain matrocliny of the hybrids which is transmitted to later generations. In v. Wettstein's (1926, 1928) crosses in mosses, this holds true especially for the shape of the leaf in the gametophyte, in Schwemmle's Oenothera crosses (1938) for the leaf shape in BI and for hypanthium length and petal size in a large number of complex heterozygotes. A number of characters in *λhh* Epilobium also are intermediate between *hirsutum* and *luteum*, such as resistance against Erisyphe and the formation of lateral branches (Michaelis, 1935b and c). Also the curvature of the stem apices shows frequent

matrocliny in Epilobium hybrids (Lehmann, 1941a and b; Lehmann and Schwemmle, 1927).

This matrocliny is especially striking when it affects the action of more or less analyzable genes. In the Habrobracon case described by Kühn (1927), the strength of pigmentation is dependent both on genes and on the plasmon. The genetic basis is shown by intermediacy of the F_1, and by the lighter coloration of the offspring from a backcross to the light parental strain. The plasmon difference is indicated by the fact that in the reciprocal crosses, in F_1 as well as in the backcross to light males, the color is always slightly but significantly lighter in cytoplasm from the light strain, than from the dark strain. The number of genes involved in this case could not be estimated.

This interaction between genes and plasmon is especially striking, when monohybrid Mendelian segregation is involved. This has been shown in Goldschmidt's (1924) studies in Lymantria races. Strains of Japanese origin have lighter larvae than European strains. If as a measurement of the extension of pigment the size of a light spot on the third thoracic segment is used, the two types are quite distinct, and their variation does not overlap. In F_1 the size of the spot is intermediate between the two strains, intergrading with both parental races. But the distribution of the reciprocal F_1's is significantly different, animals from European mothers being darker. Segregation can best be observed in backcrosses. If the hybrid ♀ Japan × ♂ Europe is backcrossed to European males, a clear bimodal curve for size of the thoracic spot is obtained, one maximum corresponding to the European type, while the other maximum, representing the heterozygotes, is darker than the Japanese race. The backcross of the reciprocal mating ♀ Europe × ♂ Japan to European males consists also of homozygotes for light color and heterozygotes, but in European cytoplasm. They have a unimodal distribution, with a maximum identical with that of the European strain; they differ from it only in the fact that about 10% of the animals are lighter than any occurring in pure European strains. In this instance, the segregation which is so obvious in Japanese cytoplasm cannot be observed in European cytoplasm, since the *εὐAa* are almost identical with the *εὐaa* animals. Similar results are found in F_2 and in backcrosses to the Japanese race. The general rule may be stated, that the same genotypes, both homozygotes and heterozygotes, are darker in European than in Japanese cytoplasm.

3. *Appearance of New Characters*

All cases mentioned until now can be described as due to a quantitative interaction of genes and plasmon. v. Wettstein (1926, 1928) has used the terms "antecedence" and "recedence" in order to designate the relative

importance of genome and plasmon for the expression of a character. In cases of completely maternal transmission the plasmon is called antecedent and the genome recedent, while in cases, where reciprocal hybrids are equal, the genome is called antecedent. Cases of interaction of genome and plasmon, like the Habrobracon and Lymantria case, would be intermediate since both genome and plasmon action can be observed. These terms, antecedent and recedent, will not be used in this review, since a large class of phenotypic plasmon expressions cannot be described in this way.

This refers to the cases in which, due to plasmon action, characters appear in the hybrid which have not been present in any one of the parental strains. The best known of these cases are the inhibitions in Epilobium, which range from pollen sterility in hybrids from pollen fertile strains to the serious inhibitions found in many hybrids in the *hirsutum* Jena plasmon (Michaelis, 1940b). Into this group would also belong the inhibitions of the complexes 1 and B in *Oenothera odorata* cytoplasm (Schwemmle *et al*, 1938), and the sterility in one sex in reciprocal crosses between *Streptocarpus Wendlandii* and *Rexii* (Oehlkers, 1938).

It may be mentioned that in the lightest cases of inhibition due to Jena plasmon, hybrid vigor in the growth of the vegetative parts has been found. That in some cases of heterosis plasmon action may be involved has been confirmed by the observations of Marshak (1934, 1936) on the growth rates of hybrids of two strains of mice. While the F_1 males of the hybrids showed heterosis but no reciprocal differences, the F_1 females from reciprocal crosses showed different growth rates, in each case higher than either parental strain. Furthermore, the regression of birth weight on litter size was different in reciprocal crosses. In backcrosses of the F_1 to the parental strains, segregation for growth genes was found, but superimposed on it there appeared a cytoplasmic effect paralleling that observed in F_1.

Of special importance for the theory of plasmon action are cases in which characters appearing in hybrids under the influence of the plasmon are due to a single segregating gene. The classical instance is male sterility in flax which has been described and analysed by Bateson and Gairdner (1921; Gairdner, 1929) and Chittenden (1927) and Chittenden and Pellew (1927). In crosses between two strains, procumbens and tall, the F_1 is normal in both reciprocals. But in F_2 from the cross ♀ procumbens × ♂ tall, 25% of the offspring are pollen sterile. No pollen sterility has been observed in F_2 of the reciprocal cross. The interpretation has been given, that the tall strain is homozygous for a recessive factor *m* for pollen sterility, which is expressed only in procumbens cytoplasm π. Therefore, only plants of the constitution π *mm* will be pollen sterile, while both possession

of the plasmon τ (tall strain) and of the allele M will cause the plant to be pollen fertile. This interpretation has been borne out by numerous backcrosses.

Similar results have been obtained by Michaelis in Epilobium (1940a). An *E. hirsutum* strain from Kew Gardens proved to be homozygous for a gene "deformans" which causes deformed, sterile flowers in the cytoplasm of some other *hirsutum* strains, *e.g.* from "Giessen" and from "Vienna" but not in "Kew" and "Munich" cytoplasm. "Deformans" plants segregate out in F_2 and backcrosses from the cross ♀ Giessen or Vienna × ♂ Kew but not in the reciprocal. Selective gametophyte or zygote mortality is excluded, since no shrunken ova were found in Kew and Munich cytoplasm, and germination was up to 100%. A similar dependence on cytoplasmic constitution and on analyzed genes has been found for male sterility in sugar beets by Owen (1942, 1945), in *Dactylis glomerata* by Myers (1946), and for a gene causing deeply slit leaves in the cytoplasm of *Streptocarpus Wendlandii* and *Comptoni* but not in that of *Rexii* (Oehlkers, 1938). While in sugar beets at least two pairs of alleles are involved together with the cytoplasm, in Dactylis there appears to be one gene, which in a suitable cytoplasm causes male sterility in the duplex, triplex and quadruplex condition, but never in the nulliplex. Plants carrying the gene in the simplex condition may or may not be male sterile. In crosses between *Nicotiana Langsdorffii* and *N. Sanderae*, East (1932) found the factorial basis to be still more complicated. The gene for self-sterility of *N. Sanderae* is involved in the production of male sterility in *Langsdorffii* cytoplasm. Furthermore, anther color, blue pollen and the production of green color in the corolla are dependent on genes acting differently in the two cytoplasms.

4. *Lethality*

Finally, there is a group of cases, in which certain chromosome or gene combinations are lethal in certain cytoplasms. In this group would belong the lethal hybrids of certain *Epilobium hirsutum* strains in Jena plasmon (Michaelis, 1942a), and the lethal combinations vv, II, Bl, and lI in *Oenothera odorata* cytoplasm which have been mentioned above (Schwemmle *et al*, 1938). In hybrids between *Drosophila simulans* and *melanogaster*, Sturtevant (1929) found that they are not viable without the presence of a *simulans* X chromosome. But also females containing a *simulans* X and *melanogaster* X chromosome in *simulans* cytoplasm (from ♀ *simulans* × ♂ *melanogaster*) show strongly reduced viability, while the same constitution is perfectly viable in the reciprocal cross. The viability of the hybrid females was extremely irregular in different strains and crosses. This result may suggest that a *melanogaster* X is lethal in *simulans*

cytoplasm. Since the hybrids are sterile, distinction between maternal and cytoplasmic inheritance is impossible in this case.

A similar case of zygotic lethality has been described by Sirks (1931a) in crosses between the subspecies *major* and *minor* of *Vicia faba*. While for a number of factors the expected segregations appeared in *major* cytoplasm, six factors showed abnormal segregations in *minor* cytoplasm. In four of these genes, the recessive which had been introduced from the *major* father failed to appear in homozygous condition in F_2. For the other two factors whose dominant alleles came from the *major* strain, a 2 :1 ratio was obtained in F_2. Since about 25% of the seeds are eliminated in the F_2 in *minor* cytoplasm, and since all the six factors showing this abnormal segregation are linked, it must be concluded that all zygotes carrying the *major* chromosome marked by the six factors mentioned in homozygous condition are killed in *minor* cytoplasm. It may be assumed (Stern, 1932), that a locus in this *major* chromosome acts as a recessive lethal in *minor* but not in *major* cytoplasm.

Cases in which spores or gametophytes carrying certain gene combinations are lethal in a foreign cytoplasm, have also been reported. This is especially obvious in the sub-family cross ♀ *Physcomitrium eurystomum* × ♂ *Physcomitrella patens* made by von Wettstein. In this case, it was possible to isolate spore tetrads. It was frequently observed that all four spores belonging to one tetrad germinated; but two of them died soon, while the other two grew up to form purely maternal or strongly matroclinous gametophytes. It is concluded that the two dying spores contained most of the paternal genes (von Wettstein, 1926, 1928a). Other cases have been reported by Sirks (1938b) in Vicia and by Skalinska (1938) in Aquilegia.

5. *Peculiarities of Cytoplasmic Differences*

A number of peculiar features of cytoplasmic effects must be mentioned. A striking number of these effects concern the haplophase. Amongst the few cases which have been worked out thoroughly, two concern organisms which possess a well developed haplophase, mosses and Habrobracon. Furthermore, the number of cases in which gamete sterility in plants is involved, is high. It must, however, be considered, that a number of cases are known in which the diplophase is affected. Furthermore, in pollen sterility, it is by no means sure that the microspore is the part originally affected. In Epilobium, at least, the diploid tapetum is the first tissue to show visible breakdown, and in higher degrees of inhibition, it is the whole anther which does not develop normally (Michaelis, 1933). In Streptocarpus, it is the diploid stamen which is transformed into a sterile staminodium or even a female pistil (Oehlkers, 1938). In Skalinska's

Aquilegia crosses, on the other hand, the spores degenerate after a normal meiosis (Skalinska, 1938).

Reciprocal differences have been observed especially in species crosses. This cannot be attributed to the assumption that cytoplasmic differences occur only between different species. As a matter of fact, the cytoplasmic differences found in Habrobracon (Kühn, 1927) and Drosophila (L'Héritier and Teissier, 1937) concern inbred strains of the same species, and the cases of Epilobium (Lehmann and Schnitzler, 1932; Michaelis, 1940b), Vicia (Sirks, 1931a, b and c), Zea (Rhoades, 1933), Lymantria (Goldschmidt, 1924) and others involve local biotypes or varieties of the same species. The reason for the more frequent finding of reciprocal differences in species hybrids seems to lie in the fact that the hybrid genome is more sensitive. Cytoplasmic differences between different strains of the same species of Epilobium, *e.g. E. hirsutum* or *E. parviflorum*, do not show up in the hybrid, but only after the hybrids have been outcrossed to a different Epilobium species (Lehmann and Schwemmle, 1927; Lehmann and Schnitzler, 1932; Brücher, 1938, 1940b). Michaelis has tried to explain this by assuming that a number of genes, those genes by which the two species differ, may be functionally haploid in the hybrid, in this way linking this phenomenon up with the frequent expression of cytoplasmic effects in the haplophase. It may be safe to state that hybrid genomes are in general more unbalanced, and that unbalanced genotypes would be more likely to show up cytoplasmic differences. An analogy would be found in the effect of pieces of the X chromosome in Drosophila which when added to a normal balanced genome do not have any effect, but push the development toward femaleness in triploid intersexes (Dobzhansky and Schultz 1931, 1933).

One feature of cytoplasmic effects should be mentioned, their great variability and dependence on the environment. The fact has been mentioned that pollen sterile maize is frequently phenotypically fertile and that the inhibitions of the hybrid ♀ *E. hirsutum* Jena × ♂ *E. hirsutum* Munich are not present at low temperatures (Michaelis, 1940). Many of the characters affected by the plasmon, such as the pigmentation in Habrobracon (Kühn, 1927) are especially sensitive to temperatures. In inhibited Epilobium hybrids it turns out that different developmental stages are affected to a very different degree by the same genotypic and cytoplasmic situation (Michaelis, 1938, 1942a). Furthermore, the phenotypic differences between *ιhh* and *λhh Epilobium hirsutum* are more pronounced under bad environmental conditions, *e.g.* when the plants are kept in small pots (Michaelis, 1935c). The pollen sterility characteristic for many *λhh* plants can be removed temporarily by treating the plants with certain substances, such as HgCl, $CuSO_4$, $NiSO_4$, $ZnSO_4$, (Michaelis

and von Dellingshausen, 1935). These are only some examples for a frequent phenomenon.

IV. The Frequency of Occurrence of Cytoplasmic Differences

The facts described make it possible to discuss the question of how frequently cytoplasmic differences are actually present. The opinion is widespread that true cytoplasmic inheritance is so rare a phenomenon, that it must be regarded as an exception of minor importance. The number of cases described, though not large, is now large enough to suggest that a normal phenomenon is actually involved which is not as easily detected as Mendelian inheritance. Therefore, some of the difficulties involved in establishing cytoplasmic differences should be discussed.

Reciprocal differences are rather frequently found in species hybrids. Although many of them have been shown to be due to maternal inheritance, others are certainly due to cytoplasmic inheritance. The differentiation between these two possibilities is frequently impossible, due to the low fertility or complete sterility encountered in species hybrids. Further difficulties arise due to the dependence of cytoplasmic effects on environmental factors. It is a frequent finding, that significant differences between the same genotypes in different plasmons are found in one year, but not in the next one, or if plants are kept in small pots, but not in the field. This peculiarity necessitates frequent repetition of the same experiment, and as careful a control of environmental factors as possible.

Furthermore, the fact that frequently cytoplasmic differences do not show up phenotypically adds to the difficulty of establishing them. It has been mentioned above, that the differences between the plasmons of different local varieties in *Epilobium hirsutum*, *parviflorum* or *roseum* do not show up in the reciprocal hybrids, but only in their outcrosses to a different Epilobium species (Lehmann and Schnitzler, 1932; Brücher, 1938, 1940b). The same has been found for species differences within the genus Epilobium by Michaelis and Wertz (1935). For instance, *Epilobium luteum* and *montanum* hybrids are reciprocally equal. This does not mean, that *montanum* and *luteum* plasmons are identical. For in reciprocal crosses of certain *montanum* strains to *λhh*, differences between the *hm* plants in *luteum* and in *montanum* cytoplasm are found which are pronounced under adverse environmental conditions.

Therefore, even where no reciprocal differences are found, plasmon differences may or may not be present. Stern, Schaeffer and Spencer (1944) transferred the whole genome of *Drosophila virilis* into the cytoplasm of *Drosophila americana*, and found that 10 phenotypic differences between the two species, some of them physiological, are all due to genes. The same is true for most of the phenotypic differences between *Epilobium*

hirsutum and *luteum*. In spite of this, plasmon differences have been demonstrated in the latter instance, which are expressed especially in inhibitions in the *hl* hybrid.

Because of all these difficulties, a human element must be considered in the evaluation of the frequency of occurrence of plasmon differences. That is the long time and the amount of work involved in establishing cytoplasmic differences beyond doubt. It is a matter of record that in two of the best investigated cases, in Michaelis' *Epilobium hirsutum* × *luteum* crosses, and in Schwemmle's Oenothera case, ten years have elapsed between the beginning of the experiments and the first extensive publication.

These considerations do not prove that plasmon differences are actually more frequent than usually assumed. But they give reasons why they may remain unobserved, when present. The possibility exists, therefore, that cytoplasmic differences may be a relatively frequent phenomenon. This may explain why in the last ten years, after genetic techniques have become more refined, a relatively large number of new cases of cytoplasmic effects have been described.

V. Plasmon and Genic Action

1. *The Theory of Plasmon-sensitive Genes*

The theory which seems to describe the different expressions of the plasmon satisfactorily is the theory of *plasmon-sensitive genes* first proposed by Renner and Kupper (1921). It assumes that the foreign cytoplasm provides an unsuitable substrate for the paternal genes, and that therefore certain specific plasmons inhibit the action of specific genes to different degrees. In this way, maternally transmitted characters would be due to the fact that the action of certain paternal genes would be completely suppressed; *e.g.* in pollen sterility in maize (Rhoades, 1933) genes necessary for normal pollen production would be completely inhibited. In Streptocarpus, genes necessary for anther formation in the species *Rexii* would be inhibited in *Wendlandii* cytoplasm (Oehlkers, 1938). In these experiments, effects attributed to single genes have been obtained in crosses involving *Streptocarpus polyanthus* (Oehlkers, 1941). The F_1 ♀ *S. Wendlandii* × ♂ *S. polyanthus* is male fertile. In F_2, 25% of the progeny have no anthers, while in the backcross to *S. polyanthus* pollen 50% of the progeny are male sterile.

Matrocliny would be due to quantitative inhibitions of distinct paternal genes. The inhibitions characteristic of many of the Epilobium crosses would be explained as inhibitions of the action of fertility and growth genes. Also the lethality of certain genes, gene complexes and

chromosomes (Vicia) in foreign cytoplasm can be easily understood under this theory.

2. *The Role of Plasmon-sensitive Genes in Inhibitions Due to the Plasmon*

Actual evidence for the existence of plasmon sensitive genes has been obtained in experiments with Epilobium. A case described by Lehmann and Schwemmle (1927; Schwemmle, 1924) will be used as an example. Two strains of *Epilobeum roseum* were crossed to *E. parviflorum.* If *p* represents the genome of *parviflorum,* and *r* that of *roseum, pr* hybrids are sterile in both sexes and have reduced flowers when the mother is *parviflorum.* This effect is stronger with one *roseum* strain, which may be called *r*I, than with the other, *r*II. In the reciprocal cross, complete sterility was also found, when *r*I was the mother. But with♀ *r*II, the *r*II*p* hybrid was fertile both as male and female, although the fertility was lower than in the parent strains. In Table 4 these results are indicated, designating with $\pi\alpha$ the plasmon of *parviflorum,* with $\mathring{\rho}_1$ and $\mathring{\rho}_2$ the plasmons of the two *roseum* strains respectively. If hybrids of the two *roseum* strains are outcrossed as ♀♀ to *parviflorum* as the pollen parent, a 1 :1 segregation into sterile plants and plants with reduced fertility is found. This is interpreted as indicating the presence of an "inhibitory" gene which causes sterility and flower reduction in hybrids with *parviflorum.* The inhibitory gene in *roseum* II is apparently less active than that in *roseum* I, causing only reduced fertility in *roseum* cytoplasm, and a lesser reduction of the flowers in *parviflorum* cytoplasm. This type of experiment has been repeated by Lehmann and his students, by Brücher and by Michaelis with many different Epilobium species, and the action of segregating genes has been definitely established.

If the fertile hybrids from the cross ♀ *r*I*r*II × ♂ *parviflorum* are

TABLE 4

Fertility of Crosses of *Epilobium parviflorum* and *roseum* (after Lehmann and Schwemmle, 1927)

Cross		Constitution	Fertility
♀ *parviflorum*	× ♂ *roseum* I.....	$\pi_\alpha\, pr_I$	Sterile
♀ *parviflorum*	× ♂ *roseum* II....	$\pi_\alpha pr_{II}$	Sterile
♀ *roseum* I	× ♂ *parviflorum* ..	$\mathring{\rho}_I pr_I$	Sterile
♀ *roseum* II	× ♂ *parviflorum* ..	$\mathring{\rho}_{II} pr_{II}$	Reduced fertile
roseum I	× *roseum* II	$\mathring{\rho}_I$ or $\mathring{\rho}_{II}$ $r_I\, r_{II}$	Completely fertile
♀ *roseum* (I × II)	× ♂ *parviflorum* ..	Combinations of $\mathring{\rho}_I pr_I$ and $\mathring{\rho}_I pr_{II}$	50% Sterile, 50% fertile

arranged according to the degree of fertility, a bimodal curve is obtained, indicating segregation for a second pair of genes influencing fertility. It is in this experiment, that differences between the plasmons of the two *roseum* strains can be demonstrated, since the most fertile plants give less seeds per capsule in the plasmon of *roseum* I, than if *roseum* II had been the mother of the hybrid. The experiment permits therefore finding cytoplasmic differences between *E. roseum* I and *E. roseum* II, by the degree of fertility of the fertile plants arising in the cross (line 6 in Table 4).

The constancy of this type of plasmon difference through four generations of backcrossing has been demonstrated by Brücher (1932) in crosses between different strains of *E. hirsutum* and *E. adenocaulon*.

In principle this result is not different from the one described for the deformans gene in *E. hirsutum* Kew, the male sterility genes in Linum, in Nicotiana, in Dactylis and sugar beets and for the genes of *Streptocarpus Rexii* causing transformation of the stamens into carpels in *S. wendlandii* cytoplasm. In all these cases, analyzable genes have been demonstrated which have different activities in a different cytoplasmic background.

That these different activities actually constitute an inactivation of genes in a foreign plasmon is most evident in certain results obtained by v. Wettstein and his students. In many species of mosses autopolyploids can be obtained by the regeneration method. If a number of genomes of the same species are placed into the same cytoplasm, the cell volume increases, with increasing number of genomes according to an exponential function (von Wettstein, 1929a; 1930); the osmotic value decreases (Becker, 1932); and the speed of cell propagation decreases in *Funaria hygrometrica*, but it increases in *Physcomitrium piriforme* protonemata (Dörries-Rüger, 1932). In hybrids between two species, balanced allopolyploids can be made by the regeneration technique, *i.e.* the series $PiHy$, Pi^2Hy^2 . . . Pi^4Hy^4 can be established. In the lower polyploids, an increase in cell volume is obtained, but in the higher members of the series it reaches a constant value which is not increased by further addition of genomes. In other words, if cell volume is plotted on the number of chromosome sets present, a hyperbolic curve is obtained.

From the point of the present discussion, the unbalanced polyploids are most interesting, *i.e.* plants which combine one or two maternal genomes with up to five paternal ones. If, for instance, the series $\pi_\iota PiHy$-$\pi_\iota PiHy^2$-$\pi_\iota PiHy^3$ is compared, the volume of the leaf cells increases with increasing addition of *Hy* genomes, but less strongly, the more *Hy* genomes were already present. If, instead of leaf cells, the volume of protonema cells is measured, a maximum volume is obtained at $\pi_\iota PiHy^2$, $\pi_\iota PiHy^3$ having smaller cells. The same is true for leaf cells, if *Physcomitrella patens* is used as mother, instead of *Physcomitrium piriforme*. It appears from these

facts, that the action of the *Hy* genome in foreign cytoplasm on cell volume seems to be diminished, especially if several genomes are present. This seems to indicate some quantitative limitation of its action in the foreign plasmon.

Similar results could be obtained by measuring the osmotic pressures of the cells (Becker, 1932). The values obtained for the osmotic values of different genetic constitutions are reciprocally proportionate to the cell volumes. Also in many morphological traits, *e.g.* peristome formation, added genomes in other than their own cytoplasm contribute less effect than the first one, or none at all. This is the more striking, since in autopolyploids two or more recessive genes frequently phenotypically suppress the expression of one dominant. All these experiments seem to indicate that the genes acting on the characters described are not fully active in foreign cytoplasm.

Similar results can also be found in Schwemmle's Oenothera experiments (Schwemmle *et al*, 1938). It has been mentioned that *odorata* has larger pollen grains than *Berteriana*, and that in the new complex heterozygotes, the two types of pollen grains segregate. In these segregations, the complex which is foreign to the cytoplasm consistently forms differently sized pollen grains than in its own cytoplasm. The lv heterozygote forms two types of pollen grains, which can be phenotypically distinguished by their size, v pollen (from *odorata*) being larger than l pollen (from *Berteriana*). But in *odorata* plasmon, the l pollen is somewhat larger than in the original *Berteriana*, while the v pollen maintains the same size as in the original *odorata*. In *Berteriana* cytoplasm, on the other hand, the l pollen remains unchanged, while the v pollen is reduced. It has been mentioned above, that this change in pollen size in foreign cytoplasm becomes more pronounced in successive generations of selfing. The same rule applies to the other complex heterozygotes tested for pollen size. Similar types of inactivation of genes in foreign cytoplasm have been obtained with vegetative characters such as leaf shape.

A thorough analysis of the plasmon sensitive genes in *Epilobium hirsutum* crosses has been made by Michaelis (1940d, 1942a). It has been stated above that different strains of *E. hirsutum*, crossed as the pollen parent to a *hirsutum* strain from Jena, gave hybrids which shared different degrees of inhibition, from heterosis to lethality, depending upon the particular strain used as the pollen parent. It has also been mentioned, that these inhibited hybrids can be arranged in a linear series of inhibitions. In order to analyze the plasmon-sensitive genes, the different strains were outcrossed as the female parent to Jena pollen, giving fertile hybrids. These hybrids, or the results of further backcrosses, were crossed to the Jena strain as female, and the inhibitions in the progeny observed.

The scheme of these crosses would be ♀ Jena × ♂ (♀ strain × ♂ Jena); in the pollen, all possible types of combinations of Jena and strain genes are expected to occur. It was found, that from the strains showing slight inhibitions in Jena plasmon, a small number of segregating plasmon-sensitive genes could be isolated. In crosses involving a strain "Vienna," 2 plasmon-sensitive genes for flower size, 3 for leaf shape and 3 for size could be estimated. In other crosses, plasmon-sensitive genes could be identified by linkage, *e.g.* to a gene causing hairy pods. In strains with higher degrees of inhibitions, the number of plasmon-sensitive genes was larger, so that analysis proved difficult or impossible. Finally, in crosses with the most inhibited strains, a whole series of different degrees of inhibition was obtained, exactly comparable to the series obtained by crossing Jena as female to different strains, and ranging from heterosis to crippled dwarfs and lethals. Morphologically, the less inhibited segregants have the greatest similarity to the Jena strains, suggesting, that the plasmon-sensitive genes come from the paternal strain.

From this series of experiments it is concluded, that the degree of inhibition in Jena plasmon is not dependent upon the quality of certain plasmon-sensitive genes, but on the quantity. A slight degree of inhibition occurs when the number of plasmon-sensitive genes is small, while in the extremely disturbed hybrids, a large number of genes of the paternal strain seem to be sensitive to Jena plasmon.

Michaelis concludes also from these experiments, that the "developmental tendency" of the Jena cytoplasm is the actual determining factor for the inhibitions, its degree being determined by the number of plasmon-sensitive genes involved, without regard to their quality. It must, however, be taken into account, that very many gene differences, besides their morphological effects most easily observed in crossing experiments, cause very definite differences in general physiological characters such as growth, speed of development and fertility. Another group of genes may affect only these quantitative characters. It is, therefore, sufficient to assume that the Jena plasmon interferes with these physiological activities of the genes. Furthermore, in some cases morphological effects of certain plasmon-sensitive genes in Jena cytoplasm have been described.

3. *Plasmon-sensitive Genes Derived from the Maternal Strain*

In this case, as well as in many of the preceding ones, the plasmon-sensitive genes were derived from the parent whose cytoplasm did not enter the hybrid. This is also true for the deformans gene in *Epilobium hirsutum* Kew (Michealis, 1940a), and the male sterility gene in flax (Bateson and Gairdner, 1921; Chittenden and Pellew, 1927). But the fact that maternal genes can also become plasmon-sensitive in hybrid

constitutions is suggested by the experiments with *Epilobium roseum* × *parviflorum* in which *roseum* genes are apparently sensitive to *roseum* cytoplasm (Lehmann and Schwemmle, 1927).

That maternal genes may also be plasmon-sensitive has been demonstrated for the *Epilobium hirsutum* strain Jena: some Jena genes may become plasmon-sensitive to Jena cytoplasm. This is best demonstrated by observations on two strains of *hirsutum* Jena derived from the same original plant which was heterozygous for a pair of alleles $parv^+$ and *parv*. In outcrosses to other strains, the strain homozygous for $parv^+$ genes consistently gives a higher degree of inhibitions than the strain homozygous for *parv*. $parv^+$ seems therefore to be more sensitive to Jena plasmon than *parv* (Michaelis, 1942c).

The way in which plasmon-sensitive genes from the two parental strains interact can be seen in successive outcrosses of *hirsutum* Jena as female to *hirsutum* Erlangen. This cross does not show any reciprocal differences in F_1, indicating that the Erlangen genes are not plasmon-sensitive. But if the F_1 ♂, (♀ Jena × ♂ Erlangen) is outcrossed to ♀ Jena, great phenotypic variability is observed in the offspring, giving many plants with the typical Jena plasmon inhibitions. In later generations of backcrosses of the hybrid ♀♀ to Erlangen ♂♂, the inhibitions in the progeny in Jena plasmon gradually disappear, as the Jena genes are replaced by Erlangen genes (Michaelis, 1942c).

In other words, neither the Jena/Erlangen hybrid genome nor the Jena/Jena and the Erlangen/Erlangen homozygotes give rise to inhibitions in Jena cytoplasm, but some of the segregations in backcrosses to Erlangen contain plasmon-sensitive genes. It must be concluded that the same genes, and that applies to Erlangen as well as Jena genes, may be plasmon-sensitive or not, depending on the remainder of the genome. This is a new formulation of a generalization made above, that hybrid genomes and unbalanced genotypic constitutions are generally more susceptible to plasmon action than homozygotes.

The same result has been obtained in outcrosses of *E. hirsutum* Jena to other *hirsutum* strains, with the important exception, that the number of generations of backcrossing from which inhibitions can be obtained is different in different crosses. While in outcrosses of ♀ Jena × ♂ Erlangen, Vienna or Munich to the paternal strain for 5 generations no inhibitions are obtained if outcrossed to ♀ Jena, the crosses of (♀ Jena × ♂ Kew) still segregate in the fifth generation for plasmon-sensitive genes. This shows, that some Jena genes are probably also present in the fifth generation of backcrossing to Erlangen, but in this case they are not plasmon-sensitive, while in combination with the Kew genes they are (Michaelis, 1942c).

The same result has been obtained by Michaelis from the study of a triploid which contained one *hirsutum* genome of strain Jena, one *hirsutum* genome of another strain, "I," and a *luteum* genome in *hirsutum* Jena cytoplasm (Michaelis, 1942b). This plant was in the first year completely uninhibited and looked like a *hirsutum* homozygote. In later years, it became more similar to an *hl* hybrid, and so was all its vegetative progeny. Its sexual progeny by selfing included both inhibited and uninhibited plants. In backcrosses to *E. luteum*, it turned out that those plants which were phenotypically similar to either *hirsutum* or *luteum* were uninhibited, while the plants which showed more hybrid characters were most strongly inhibited. It is suggested that in spite of the fact that the *hl* hybrid is severely disturbed in the F_1, and could be backcrossed to *luteum* only up to the third generation (see page 9) the *ll* homozygote may be viable in *hirsutum* cytoplasm, and that the *luteum* genes are plasmon-sensitive only when in heterozygous condition with *hirsutum* genes.

All these experiments lead to the conclusion, that plasmon-sensitivity is not a constant character of particular genes, but that the plasmon-sensitivity of the genes depends upon the presence of other genes in the same cytoplasm. The same gene may or may not be sensitive towards the same cytoplasm, depending on other genes present in the genome.

In Paramecium, Sonneborn (1943a and b, 1945a, and 1946) described a character "killer" which is dependent in its expression on both a gene and a cytoplasmic factor. The character consists in the excretion of a substance which is able to kill non-secreting strains. All non-secreting strains are sensitive to the killer substance. The formation of killer substance is due to a dominant gene *K*, its recessive allele *k* causing sensitivity.

In crosses between a *KK* and a *kk* strain, the heterozygote which contains the cytoplasm of the original "killer" animal produces "killer" substance, while the other exconjugant does not and is sensitive, in spite of the presence of the gene *K*. It is concluded, that a cytoplasmic product κ (kappa) is necessary for the action of the gene *K*; therefore, even homozygous *KK* individuals cannot form "killer" substance, if κ is absent from the cytoplasm. This interpretation has been well established by a large amount of supporting evidence.

While Sonneborn first assumed, that the cytoplasmic κ factor is a product or replica of the gene *K*, he has proposed recently (Preer, 1946; Sonneborn, 1945b) that it may represent an independent cytoplasmic constituent which gradually disappears in absence of the gene *K*. This interpretation falls in line with the theory of plasmon-sensitive genes, since the action of the gene *K* would be inhibited by non-κ cytoplasm. The loss of κ in the absence of *K* is parallel to the plasmon changes in the presence of

foreign genes in Epilobium (Michaelis, 1933) and Nicotiana (Honing, 1930, 1932).

VI. The Mechanism of the Inhibition of Plasmon-Sensitive Genes

If a theory of plasmon action is attempted, two methods of approach are open. Either physiological differences between organisms having the same genome but different plasmons may be compared, and conclusions drawn as to the probable developmental mechanisms causing their differences. Or the action of plasmon-sensitive genes may be interpreted in terms of the theory of gene action. Although all of the experimental attempts have been following the first method, the second one will be discussed first.

1. *Plasmon-sensitive Genes and the Theory of Gene Action*

In order to arrange the facts about plasmon-sensitive genes into the theory of gene action, a number of hypotheses may be proposed. It should be mentioned that these hypotheses are not mutually exclusive.

It could be imagined, that plasmon-sensitive genes are inactivated in the foreign plasmon by immunological reactions. The assumption of immunological reactions in questions of gene action and gene multiplication has frequently been made. But since no factual material is available, it seems futile to discuss this hypothesis at present.

In the field of genetics, the phenomenon most nearly related to the cytoplasmic effects appears to be the position effect. In both cases, the action of a gene is dependent on its intracellular environment, on its intrachromosomal environment in position effects, on the cytoplasm and on the other chromosomes in cytoplasmic inheritance. In both phenomena it is obvious that the action of a gene is not autonomous, but influenced by its intracellular environment.

This similarity between plasmon-sensitive gene action and position effect is borne out by the fact that similar interpretations for the two phenomena have been proposed independently by Stern (1943, 1944; Stern and Schaeffer, 1943a and b) and by Michaelis (1942b). In interpreting the action of the gene *ci* in Drosophila, Stern assumes, that the primary action of a gene consists in the transformation of a substrate S which is available in limited amounts into a product P. The position effect is explained by larger or smaller amounts of substrate available to the gene in different positions, and by competition of the two alleles for the limited amount of substrate.

It may be permissible to assume that the substrate or its precursor is ultimately derived from the cytoplasm. If then a certain cytoplasm

does not produce a substrate suitable for a certain gene of a different species, this gene would not have the opportunity to exert its influence and appear plasmon-sensitive. In the mosses studied by v. Wettstein and his collaborators, it has been found that certain characters such as cell volume and osmotic value are influenced by *one* foreign genome, but that more than one foreign genome introduced into the cell may be without effect (von Wettstein, 1928a; Becker, 1932). This seems to suggest that a limiting factor for the action of the foreign genes is present in the cytoplasm, and it is a simple assumption that the genic substrate is changed quantitatively and qualitatively.

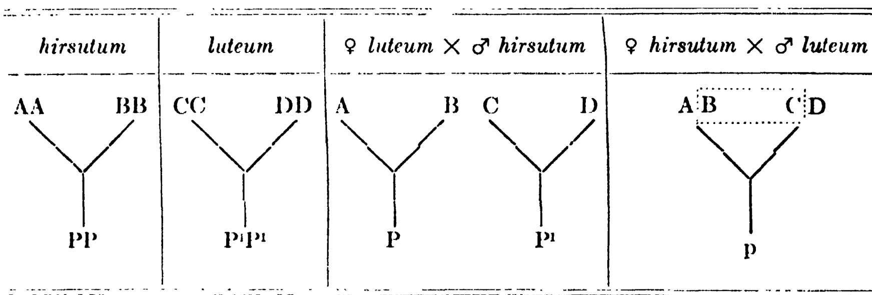
Certain difficulties for this hypothesis seem to arise from the fact, that, in the case of Epilobium, Jena genes themselves may appear plasmon-sensitive in Jena cytoplasm. But this can be explained along lines Stern has used for the explanation of factor interaction. If each allele has a characteristic combining power with the substrate, and a characteristic efficiency in transforming it to the product, it seems likely, that the two alleles will be in competition with each other for the limited amount of substrate, and that the amount of product finally produced will depend on the outcome of this competition. Assuming, that the substrate produced in the cytoplasm of two related strains is slightly different, and that the foreign gene has a higher combining power but a lower efficiency toward this particular substrate or transforms it into a physiologically inactive product, the following situation would prevail. In the hybrid, the foreign allele would obtain more of the limited substrate, because of its higher combining power, and the allele belonging to the strain from which the cytoplasm is derived would therefore obtain less substrate and produce less product than ordinarily. The cytoplasm-foreign gene would also form less or no product, because of its lower efficiency, or because it forms a different, inactive product. Therefore, a reduced amount of active product would be present in the hybrid. In this case, the gene derived from the strain from which the plasmon was obtained would appear plasmon-sensitive.

Michaelis (1942b) has proposed a formally similar hypothesis. The difference between his hypothesis and the one discussed previously consists in the assumption, that the reactions involved take place between gene products and that two gene produced substances have to combine to give one physiologically active product. The theory is represented schematically in Table 5. It is assumed, that in *E. hirsutum* two gene produced substances, A and B, give rise to a product P, in *luteum* two different gene produced substances, C and D, give rise to a physiologically equivalent product P¹. In the cross ♀ *luteum* × ♂ *hirsutum*, both P and P¹ are formed. But the *hirsutum* cytoplasm is so constituted, that the

substrate B produced by the *hirsutum* genes and C produced by *luteum* genes form an inactive compound p. The consequent lack of P and P^1 results in the inhibitions. This theory is able to explain that plants carrying a large number of *luteum* genes in *hirsutum* plasmon are less inhibited

TABLE 5

Schematic Representation for the Action of the Plasmon on Plasmon-sensitive Genes (after Michaelis, 1942)



than the F_1 heterozygote. For they will produce mainly A and B, and no C is produced which could combine with B. Under this hypothesis it must be assumed, that every time a paternal gene appears inhibited, a maternal one would be too. This is in agreement with the fact, that while both Erlangen and Jena genomes in homozygous condition are not inhibited in Jena cytoplasm, some genes in heterozygous condition are (Michaelis, 1942c). In other cases, where only distinct paternal genes are inhibited (Linum, deformans in Epilobium, Streptocarpus), a form of the simple theory of lack of substrate or of destruction of a gene product would have to be applied.

A quite different approach has been suggested by Correns (1928). Correns proposed that the developmental processes characteristic for any species, and their normal integration in time is dependent upon the cytoplasm, and that the genes interact with it quantitatively at certain specified times and places. This theory assumes therefore, that the genes are called into action during development at different times and places, and that they are otherwise inactive. This developmental activation and inactivation of genes would be due to regularly proceeding developmental changes in the cytoplasm.

This idea presupposes, that the action of genes during development is limited in time. This has certainly been frequently assumed, but the possibility that every gene acts constantly in every cell of the body has not been excluded. The evidence pertinent to this question has been

discussed by Stern (1940). It is concluded in this paper, that a distinction between the two possibilities is not possible at present. There is, however, some evidence indicating, that continuous gene action throughout development may occur. This seems to be the case in the gene *a* in Ephestia which causes red eyes and other reductions of pigment both in the larva and in the adult (Caspari, 1933, 1946). It could be demonstrated that this defect in pigment is due to lack of a pigment precursor, Kynurenin, and that Kynurenin is produced in a^+ animals in all organs investigated, while it is lacking in *aa*. In this case, the gene a^+ certainly exerts its characteristic activity from embryonic stages on, through larva and pupa until the imago, and furthermore, at least in the larva, in all types of tissues investigated. Stern (1940) comes to the same conclusion with regard to many lethal deficiencies in Drosophila which have been shown by Demerec (1934, 1936) to be cell lethals.

A number of cases in which genes act in a particular place and at a particular time has been quoted by Hadorn (1945b). But all of them are open to the objection, that the gene may very well be active in other tissues too, without giving any phenotypical effect due to the particular developmental situation. That true inactivity of genes may occur is however suggested by Lindegren and Spiegelman's investigations in yeasts (Lindegren, 1945; Spiegelman, 1945 and 1946; Spiegelman and Lindegren, 1945a; Spiegelman *et al*, 1944, 1945). The formation of certain enzymes like Galactase and Melibiozymase is dependent on the presence of certain segregating genes. If proper alleles are not present, the corresponding enzymes cannot be formed under any conditions. But in the presence of these genes, the enzymes are formed only if their specific substrate, *i.e.* Galactose and Melibiose respectively, is present in the medium. This is an instance where a particular gene does not exert its activity unless the environmental conditions are suitable. Similar effects may well occur in the normal development of plants and animals. The objection remains, however, that the genes responsible for the formation of Galactase and Melibiozymase are still active in absence of their specific substrates, causing the formation of inactive compounds.

The possibility that gene inhibition by the cytoplasmic conditions in development is essentially similar to that of plasmon inhibition, must therefore be admitted. Michaelis has used this assumption for the interpretation of his results with hybrids in Jena cytoplasm. It is indeed impressive to observe, how the same types of inhibitions occur over and over again in F_1 hybrids of Jena with other strains in Jena cytoplasm, and in the segregants of hybrids of Jena and other strains, if backcrossed to Jena, *i.e.* in plants of presumably very different genetic constitutions (Michaelis, 1940d, 1942a). Michaelis talks in this connection of a "developmental

tendency" of the Jena plasmon which is more or less strongly developed according to the genes present.

Even if this developmental analogy for plasmon action is correct, the mechanism of gene inactivation both in development and by the plasmon must have a physical or chemical basis. Correns' theory is therefore not opposed to the analogy with the position effect and to the physiological analogies proposed above, but requires one of these, or an interpretation of a similar type as a mechanism. On the other hand, these mechanisms could still be valid even if Correns' ideas about the primacy of the cytoplasm in developmental processes are wrong.

2. *Physiological Mechanisms of Plasmon Differences*

A number of attempts have been made to describe more definitely the different states of the plasmon and their influence on the phenotype in physiological terms. Schlösser crossed two strains of the wild tomato *Lycopersicum esculentum*, and obtained strong matrocliny in the reciprocal crosses in F_1. In F_2, he obtained both matrocliny and segregation into two types, of which the smaller one made up only one-fourth of the total number. He concluded that two factors are involved in the determination of size, one pair of genes and the strain specific plasmons. A third factor is environmental conditions, dry culture conditions tending to increase the matrocliny in F_1 and F_2.

The interaction of these three factors conforms to the following hypothesis: the final growth attained by the plant depends upon the osmotic value and the pair of genes which cause a certain growth at a certain osmotic value. This does not mean that genes do not influence the osmotic value in the cells themselves; but in the two strains used, the genes influencing osmotic pressure are identical and it is dependent on plasmon and environment only, a certain osmotic value being transmitted purely maternally, culture conditions being constant. Schlösser interprets these results to mean that the growth genes involved have an optimum osmotic pressure for their activity, and that they are less active outside this range.

von Dellingshausen compared *E. hirsutum* (*ihh*) and *E. hirsutum* with *luteum* plasmon ($\lambda_{12}hh$) for a number of cell physiological characters. No consistent difference was found for osmotic values. But the permeability for glycerine, KCl and the lipid soluble succinimide as measured by the time needed for deplasmolysis after preceding plasmolysis, was higher in λhh than in *hirsutum*. This would indicate differences in the chemical and physical constitution of the cell membrane. Furthermore, the viscosity of the cytoplasm, as measured by the outline of the protoplast at plasmolysis, and by the displacement of chloroplasts at centrifugation, is higher in *hirsutum* than in λhh.

A connection of these facts with the morphological effects of *luteum* plasmon may be derived from the action of chloral hydrate. Treatment of plants with chloral hydrate decreases pollen fertility, in λ*hh* more than in pure *hirsutum*. Similarly, chloral hydrate increases viscosity in *hirsutum*, and decreases it in λ*hh*. The increase of viscosity in *hirsutum* under the influence of chloral hydrate decreases its permeability to urea. Hence, it is regarded as indicating higher resistance which expresses itself in lesser damage to pollen fertility. Similarly, the greater permeability of λ*hh* for different substances may explain its better resistance against bad nutrition.

More definite are the conclusions which have been drawn from experiments with inhibited *hirsutum* hybrids. Lehmann (1936b) suggested first, that the inhibitions may be due to lack of growth hormones, and his student Hinderer (1936) was able to prove by avena coleoptile tests, that the vegetation points of strongly inhibited *hirsutum/adenocaulon* hybrids contain less growth hormone than those of weakly inhibited or uninhibited ones. The analysis was continued by Ross (1940), using different more or less inhibited *hirsutum* hybrids in *hirsutum* Jena plasmon. He could remove a number of the inhibition characters by spraying the vegetation points with heteroauxin. As a result of this treatment, the plants lost their stunted appearance, the internodes became longer, the stem heavier, and the leaves lost their wavy appearance and the excessive weight characteristic of inhibited hybrids. Since application of heteroauxin to the leaves was without effect, even on the leaves themselves, it is concluded that the excessive weight and irregular shape of the leaves of inhibited hybrids is due to storage of substances normally used for growth of the shoot.

It must be emphasized, that certain characters peculiar to the inhibitions are maintained in the heteroauxin treated plants, such as the deficiency in anthocyan, and the fine mottling of the rosette leaves. There are, therefore, two types of inhibition characters, auxin sensitive and auxin indifferent ones.

In a further series of experiments, Ross (1941) tested the dissimilatory metabolism in 15 different hybrids in Jena plasmon, and their uninhibited reciprocals. It was found, that the activity of oxidizing enzymes, especially peroxidase activity, in the inhibited hybrids in Jena plasmon is always higher than in their uninhibited reciprocals, and that the difference in peroxidase activity is directly proportional to the strength of inhibition in the hybrid.

This result permits the conclusion that all inhibitions in Jena plasmon may be due to the increased peroxidase activity. The excessive weight and size of the leaves in inhibited plants would tend to suggest, that the amount of auxin formed is normal, and that it is more rapidly decomposed during its migration, due to increased peroxidase activity. The auxin

indifferent characters may be due to the direct action of peroxidase in the cells. Ross finally suggests, that the higher peroxidase activity in inhibited hybrids may be due to a different degree of solvation of the plasmon colloids. Other results of Ross (1941 and quoted by Michaelis, 1942a) seem to indicate, that the increase in oxidative activity in inhibited hybrids leads also to a reduction of the carbohydrate reserves which in turn may lead to developmental disturbances. This may explain the fact, that inhibitions are less pronounced at low metabolic intensity, *e.g.* at low temperature, in runners and at the rosette stage, than at stages with high metabolic activity.

These results may also give some indication about the action of plasmon-sensitive genes. Since auxin metabolism, respiration and carbohydrate metabolism are influenced by the particular constitution of the Jena plasmon, many genes which have an action on these fundamental processes will appear changed in their phenotypic expression, *i.e.* be plasmon-sensitive.

Whichever of the proposed mechanisms of plasmon activity may turn out to be correct, it is obvious now, that they are not at variance with present theories of gene action, and that they may offer a novel approach for the study of this problem.

VII. The Influence of the Plasmon on the Genome

It has been shown previously, that changes of the plasmon under the influence of a foreign genome have been observed in numerous cases. There exists also some evidence that a genome may change under the influence of a foreign plasmon. The evidence for this thesis will be discussed in the present chapter.

1. *Genome Changes in Epilobium*

The possibility of a change of a genome under the influence of the plasmon has first been suggested by Schmidt (1932) on the basis of a finding of Michaelis (1933). This result consists in the fact, that λ*lh* hybrids are somewhat more fertile, when they have been derived from a cross ♀ *luteum* × ♂ (*hirsutum* with *luteum* plasmon) (λ*hh*), than if they are derived from the cross ♀ *luteum* × ♂ *hirsutum*. Since both the λ plasmon and the *l* genome in both cases are derived from *luteum*, the difference is supposed to be due to the *h* genome from the father, which in the first case has remained in a *luteum* plasmon for several generations.

Michaelis (1933) does not accept this conclusion, and points to two other possibilities, transmission of a small amount of the *luteum* cytoplasm changed by contact with the *hirsutum* genome, or a non-genic nuclear change, *e.g.* change in nuclear enzymes. Since transmission of cytoplasmic

characters through the male gamete is well established, this experiment cannot be taken as evidence for change of the genome.

2. *Transmission of Plasmon through the Male*

Though the amount of cytoplasm transmitted by the male gamete is small, there still is a certain amount which may occasionally transmit cytoplasmic characters. This would be expected to occur rather frequently if plasmon characters should be connected with certain particulate cytoplasmic constituents, such as the mitochondria, which are frequently transmitted by both male and female gametes.

Actually, there exists evidence, that transmission through the cytoplasm of the sperm is possible. Mampell (1945) has described a case in which a gene product of a mutation-inducing gene in Drosophila is transmitted through the cytoplasm of the sperm.

In some of the cases of cytoplasmic inheritance described above, evidence for transmission of the plasmon by the sperm is convincing. L'Héritier and Teissier (1937, 1938b) have found that a varying percentage of the offspring from a cross ♀ CO_2 resistant × ♂ CO_2 susceptible in Drosophila is susceptible. The percentage of susceptible progeny varies between 10% and 90%, if susceptibility is transmitted by the sperm.

Similarly, plasmon transmission by the sperm has been well established by Goldschmidt (1924) in his studies on the inheritance of pigmentation of European and Japanese Lymantria. It has been stated above that he found the difference between the two varieties to be caused by one pair of genes, and that the expression of the genes is influenced by the cytoplasm, *εὐ* cytoplasm (European) causing the genes to cause smaller thoracic spot, than *ἰαπ* (Japanese) plasmon does with the same genotype. Transmission of the plasmon by the sperm is suggested by differences in reciprocal F_2's. The evidence from one of his crosses, Berlin × Kumamoto, is given in Table 6.

Table 6 gives the outcome of different crosses involving two strains from Berlin (*εὐaa*) and Kumamoto (*ἰαπAA*). The size of the thoracic spot is measured by nine arbitrarily chosen classes, 1 being very dark, 9 the lightest class. The number in every column indicates the mean pigmentation for every one of the three genotypes. While they are well distinct in some crosses, the distribution is somewhat arbitrary in cases of intergrading variation, and the mean values in these cases are only approximate.

The conclusions, however, are unequivocal. All genotypes having *εὐ* plasmon from the mother (lines 3, 5, 6) are darker than those having *ἰαπ* plasmon from the mother (lines 4, 7, 8). In other words, the lightening action of *A* is enhanced by *ἰαπ*, somewhat inhibited by *εὐ*.

From the point of view of transmission of plasmon through the sperm, it is necessary to compare the same genotypes, derived from the same type of mother, but from reciprocally different fathers, *i.e.* to compare line 5 and 6, and line 7 and 8. It is seen, that in both cases differences

TABLE 6

Size of Thoracic Spot at the Time of the Fourth Molt in Crosses of Lymantria Strain from Berlin (εὐ *aa*) and Kumamoto (ἰαπ *AA*) in Classes (from Goldschmidt, 1924, page 267)

Cross Berlin (B) Kumamoto (K)	Formula of Parents *aa* *AA*	Progeny in Classes *aa* 1	 *Aa*	 *AA* 8.7
F_1 ♀ B × ♂ K	♀ εὐ *aa* × ♂ ἰαπ *AA*		4.32	
F_1 ♀ K × ♂ B	♀ ἰαπ *AA* × ♂ εὐ *aa*		4.59	
F_2 ♀ (♀ B × ♂ K) × ♂ (♀ B × ♂ K)	♀ εὐ *Aa* × ♂ εὐ *Aa*	1.43	4.48	8.49
F_2 ♀ (♀ B × ♂ K) × ♂ (♀ K × ♂ B)	♀ εὐ *Aa* × ♂ ἰαπ *Aa*	1.00	3.50	7.29
F_2 ♀ (♀ K × ♂ B) × ♂ (♀ K × ♂ B)	♀ ἰαπ *Aa* × ♂ ἰαπ *Aa*	2.42	5.26	8.70
F_2 ♀ (♀ K × ♂ B) × ♂ (♀ B × ♂ K)	♀ ἰαπ *Aa* × ♂ εὐ *Aa*	2.10	4.79	7.80

are found, and these differences seem to be real, since they have been obtained consistently in a large number of independent crosses, using different European and Japanese strains. It is certainly not unimportant for the phenotype of the progeny, from which strain the cytoplasm of its father was derived. Under the assumption, that εὐ plasmon inhibits the action of gene *A*, it can be easily understood, that paternal εὐ cytoplasm, if added to maternal ἰαπ cytoplasm, causes darker larvae (line 7 and 8). But it is difficult to understand, that in line 5 and 6, ἰαπ plasmon introduced by the father causes a darker phenotype than εὐ plasmon introduced by the father. Goldschmidt expresses these facts in the generalization, that the more mixed the cytoplasm is, the more does it tend to inhibit the gene *A*. While the mechanism of the plasmon effects transmitted by the father remains doubtful, there is no doubt about the consistent differences between double reciprocal crosses, indicating an influence of the paternal plasmon. This has also been borne out by numerous backcrosses.

In Epilobium, on the other hand, Michaelis (1935a) assumes, by analogy to plastid transmission from the father, that only in 0.2% of the cases plasmon is transmitted through the pollen. Rhoades (1933) could not find any transmission of male sterility by the occasional pollen of partly fertile maize.

There appears to exist a certain variability in the transmission of plasmon characters through the male gamete. At all events it is not excluded, so that Schmidt's assumption of a change of the *hirsutum* genome under the influence of the *luteum* plasmon must appear doubtful, but possible.

3. *Genome Changes in Oenothera*

The evidence for such a change is much better in Schwemmle's crosses of *Oenothera Berteriana* × *odorata* (Schwemmle *et al*, 1938). This change in genome has been detected in analyzing the fact, that in the complexes BI, 1v, Bv, and vI *in Berteriana* cytoplasm, and BI and Bv in *odorata* cytoplasm, the hypanthia became consistently shorter, and the petals smaller in successive generations of selfing.

TABLE 7

Average Hypanthium Length in vI Obtained from Different Crosses
(v, I Complexes of *Oe. odorata*, *δδ* Plasmon of *odorata*, *βεp* Plasmon of *Berteriana*)
From Schwemmle, 1938, Figure 39

Cross	Average Hypanthium Length
δδvI selfed	3.47 ± 0.01
♀ δδvI × ♂ δδ1vF_4	3.41 ± 0.01
♀ δδvI × ♂ βερ1vF_1	3.43 ± 0.01
♀ δδvI × ♂ βερ1vF_5	3.14 ± 0.005

That this effect is due to a slow progressive change in the plasmon foreign genome, has been shown by crosses of the type indicated in Table 7. The same genotype from different crosses is compared. The complex derived from the mother is always identical, since for all different crosses different flowers of the same plant are used as the pistilate parent. Since they are also reared in the same year, environmental conditions will be as similar as possible. Any difference found will therefore be due to differences contributed by the pollen parent.

In all vI plants considered in Table 7, the I complex comes from the mother, the v complex from the father. It is apparent, that no real differences exist in the first three rows of Table 7. That means, that the v complex from 1v in *odorata* cytoplasm, and from the first generation of 1v in *Berteriana* cytoplasm cause the same hypanthium length as the v complex of the original *Oenothera odorata*. But, when the 1v complex heterozygote has been kept by selfing for 5 generations in *Berteriana* cytoplasm, the v complex causes significantly shorter hypanthia in the resulting vI in *odorata* cytoplasm.

A very impressive amount of data has been collected which proves

this same point over and over again. Different complexes have been studied in different combinations with the crossing technique indicated in Table 7. The result has been consistently, that complexes kept for a number of generations in foreign cytoplasm were gradually changed, while they did not change if kept for any length of generations in a combination with any other complex in their original cytoplasm. For example, v is changed in $\beta\epsilon\rho$lv, but not in $\delta\delta$lv, I in $\beta\epsilon\rho$vI, but not in $\delta\delta$vI (*odorata*), 1 in $\delta\delta$lv, but not in $\beta\epsilon\rho$lv, B in $\delta\delta$BI, but not in $\beta\epsilon\rho$BI. This explains, why the vI (*odorata* genome) has shorter hypanthia in *Berteriana* cytoplasm than the original *Oe. odorata*. It should be emphasized that in all combinations, the change observed in every complex tends to shorten the hypanthium.

Similarly, the change of complexes in foreign plasmons expresses itself in a progressive reduction in the size of the petals, and in a tendency for reduction in size in v and I pollen in *Berteriana* plasmon, but for increase in size in B and 1 pollen in *odorata* plasmon.

A similar change of genomes was found to be responsible for the recovery of the 1v and BI in *odorata* cytoplasm. These complexes, if originally obtained from the cross ♀ *odorata* × ♂ *Berteriana*, are very weak and small. The chloroplasts are reduced in number and in chlorophyll content, and very large, giving the whole plant a yellowish color. Meiosis is disturbed, and therefore fertility is strongly reduced. In successive generations of selfing, these disturbances disappear gradually, so that the fifth generation is fully green and fertile, like the same genotypes in *Berteriana* cytoplasm. The disturbances appear to be due to peculiarities of the *odorata* plastids which do not develop well with the genotypes 1v and BI. This is borne out by the fact that they cause lethality in other combinations, 1I, vv, II, B1, which are perfectly viable with *Berteriana* plastids.

The fact that the recovery is not due to a change in the plastids, is shown by pollinating a recovered BI plant with *odorata* plasmon with pollen from a 1II plant. Since the II complex is not transmitted by the pollen, two complex heterozygotes are expected, B1 and 1I. Both are lethal with *odorata* plastids, and actually no offspring was obtained from this cross, indicating that the plastids in the recovered 1v were still typical *odorata* plastids. If in the same cross, BI in *Berteriana* cytoplasm is used as mother (♀ $\beta\epsilon\rho$BI × ♂ 1II), 95% of the seeds give progeny, both B1 and 1I which are perfectly viable with *Berteriana* plastids. It has also been possible to exclude a change in the plasmon as responsible for recovery.

It must be concluded from the crosses represented in Table 8 that actually the genomes change. The degree of recovery is indicated by four steps to which the numbers 1–4 have been assigned. 4 is completely

recovered, while 1 represents the strongest degree of inhibition, the one found in F_1. Different flowers of the same individual plants were used as pistilate parent for all three different crosses. The fathers in corresponding crosses were also the same individuals.

TABLE 8

Degree of Recovery of 1v and BI from Different Crosses (after Schwemmle, 1938)

Line	Heterozygote Tested	Cross	Degree of Recovery
1	1v	♀ ὁδlvF_1 × ♂ βερBl	1
2		♀ ὁδlvF_1 × ♂ ὁδvI	2
3		♀ ὁδlvF_1 × ♂ βερvIF_7	3
4		♀ ὁδlvF_6 × ♂ βερBl	2
5		♀ ὁδlvF_6 × ♂ ὁδvI	3
6		♀ ὁδlvF_6 × ♂ βερvIF_7	4
7	BI	♀ ὁδBIF_1 × ♂ βερBlI	1
8		♀ ὁδBIF_1 × ♂ ὁδvI	1
9		♀ ὁδBIF_1 × ♂ βερvIF_8	3
10		♀ ὁδBIF_9 × ♂ βερBlI	3
11		♀ ὁδBIF_9 × ♂ ὁδvI	2
12		♀ ὁδBIF_9 × ♂ βερvIF_8	4

In line 1 and 4 in Table 8, the 1 complex of the mother is replaced by the same 1 complex from *Berteriana*. Since the two are identical, the difference in recovery between cross 1 and 4 must be due to differences in the v complexes, the one in line 4 having stayed for 5 generations as 1v in *odorata* cytoplasm. Similarly, if line 2 and 5 are compared, evidence for a change of the 1 complex in 5 generations in *odorata* cytoplasm is obtained. Comparison of line 2 and 3, and of line 5 and 6 give evidence for a change in v in *Berteriana* cytoplasm. Similar results are obtained for changes of B and I in BI (line 7-12).

These experiments indicate that gene complexes may change both under the influence of the plasmon (or plastids) and under the influence of the other gene complex. The change of v and I in βερvI, as indicated by the comparison of lines 2-3, 5-6, 8-9, 11-12 must be due to its presence in *Berteriana* cytoplasm, since vI are the natural *odorata* complexes. The changes of v in 1v with *odorata* plasmon (line 5) and of I in BI in *odorata* plasmon (line 10) must be due to association with the complexes 1 and B, since both v and I are here in their native *odorata* cytoplasm. This latter

change can also be seen from the fact, that BI in *odorata* cytoplasm in later generations of inbreeding yields a new type of progeny by selfing, which has been identified as II with *odorata* cytoplasm. This homozygote is lethal, as long as the I complex remains in its original state.

The evidence for a change in genomes in the Oenothera crosses is very suggestive, since it is derived from the observation of many different characters, and from a large number of independent experiments. The mechanism of these changes is not so clear. Renner (1937) has proposed, that the genome changes leading to recovery may be due to crossing-over between the complexes and selection for the most viable plants.

This can be easily imagined, especially since crossing-over, although rare or absent in the natural complex heterozygotes, is not infrequent in the synthetic ones. Therefore, Schwemmle has thoroughly investigated all evidence for segregation in his crosses, especially with respect to hypanthium length and petal size. Segregation for single factors influencing hypanthium length was actually found in crosses involving *Oenothera longissima*, and for multiple factors, as indicated by an increase in variance of F_2 over F_1, in crosses involving *Oe. argentinea.* But in the *odorata/Berteriana* crosses, no evidence for segregation of factors influencing hypanthium length or petal size has been found.

Schwemmle gives also some positive evidence, that the complex changes cannot be due to crossing-over. In the recovery experiment, both the v and the I complex appear recovered after 7-8 generations in *Berteriana* cytoplasm. Since v and I are the complexes of the original *odorata*, the change must be due to the *Berteriana* plasmon, since otherwise similar crossing-over should occur in *Oe. odorata.*

Similar considerations can be applied to the shortening of the hypanthia by complexes which have been in foreign cytoplasm for several generations. I for instance carries genes for very long hypanthia, while B carries genes for short hypanthia. The shortening action of I after having been in $\beta\epsilon\rho$BI for some generations can therefore be explained by exchange of factors with B; but the excess shortening action of B after having been in $\dot{o}\delta$BI cannot be explained in this way, since I contains mainly factors for long hypanthia. Similarly, the action of v in *Berteriana* cytoplasm becomes increasingly shortening for the hypanthia, independently on whether it is in the combination Iv, vI, vII, or vv. Finally, the influence of the plasmon is evident from the fact, that v and I change only in *Berteriana* cytoplasm.

Another possibility which must be borne in mind is the occurrence of mutations, and a strong selection for mutations favoring recovery and, for some unknown physiological reason, for short hypanthia. The process,

however, appears different from the usual mutation picture, because it is gradual and directed. In order to obtain observable recovery in F_2 already, the mutation rate would have to be very high. That the recovery effect is probably not due to mutation, is best demonstrated by the behavior of a recovered complex in its original constitution. The ὀδvI (*odorata*) derived from a cross ὀδvI (*odorata*) × ὀδBIF_X (recovered) contains an I complex which has been changed by several generations in the combination BI. The seedlings of this constitution are at first very weak and have pale cotyledons, but they recover during development and give rise to healthy plants. In this case, the change of the I complex back to normal can certainly not be due to selection.

Schwemmle is therefore inclined to discount factor exchange and selection as an explanation for his observations. He interprets his results on the basis of the theory of gene inactivation. It is assumed, that every complex contains growth genes for hypanthium and petals which are inhibited in foreign cytoplasm, causing in every event a shorter hypanthium. B and 1 carry genes for smaller pollen, v and I for large pollen, which are inhibited in *odorata* or *Berteriana* cytoplasm respectively. Similarly, the matrocliny in leaf shape of BI can be explained as caused by inhibition of the action of the cytoplasm foreign genes.

The only difference of this hypothesis to the general theory of plasmon-sensitive genes is the gradual increase of the effect up to a certain limit. A physiological interpretation of this fact has not yet been given.

The gradual change observed during recovery of 1v and BI in *odorata* cytoplasm introduces still another variable, the complex belonging to the cytoplasm. The combinations 1v and BI are considered "unbalanced" in presence of *odorata* plastids, and adjusting themselves gradually to this state. Changes in the plastids and in the cytoplasm could not be demonstrated, as indicated above. Experiments demonstrating, that the cytoplasm is not involved in the inhibitions and in recovery will be discussed in a later chapter.

Whatever the final physiological explanation may be, Schwemmle's experiments have demonstrated a type of change of genomes which is different from the common one by mutations and chromosome arrangements in so far as it is gradually progressing and directed. There is, however, no reason to assume that any changes in genes are involved. It would certainly be more in keeping with well established theories to consider the observed effects as results of quantitatively different action of the same genes. If considered in this way, they lend support to the consideration developed from other cases of cytoplasmic effects, that the activity of genes is influenced by states of the cytoplasm and by the plastids just as much as by the external environment.

VIII. The Relation of Plasmon and Cytoplasmic Constituents

In this chapter will be discussed the question whether the effects described in the earlier sections under the term "plasmon" may be ascribed to certain well-known cytoplasmic constituents. The following three have been proposed in the literature: plastids, viruses, and self-reproducible gene products which have been called plasmagenes (Wright, 1941, 1945; Spiegelman, 1946) or cytogenes (Lindegren, 1945). All these cytoplasmic constituents have in common, that they are particulate. The arguments for and against a particulate nature of the plasmon will therefore be discussed first.

1. *The Question of Plasmon Particles*

Not very much actual evidence is present concerning the possibility of the plasmon being corpusculate. v. Wettstein (1935, 1937) expressed the opinion that the plasmon is the aggregate of characteristics of the cytoplasm which furnishes the substrate for gene action. Schlösser's maternally transmitted osmotic value of the tomato cell (1935), and Ross' characteristically increased peroxidase activity of the *E. hirsutum* Jena cytoplasm (1940) seem certainly to favor such a view. Rhoades could not find any relation between kernel position in the ear and male sterility of the plants developing from the kernels (1933).

Darlington (1939, 1944) on the other hand, upholds the corpusculate theory of the plasmon. He bases his arguments on certain results of Michaelis (1933) which he interprets as indicative of cytoplasmic segregation. Sterile λhh plants have been found to produce occasionally fertile shoots. Since this occurs with the approximate frequency of plastid segregation for paternally transmitted albomaculatus plastids (0.2%), it is concluded that it is due to paternally transmitted *hirsutum* plasmon particles which happened to segregate out. It must be taken into account that the basis for this argument is formed by Michaelis' hypothesis, that the difference of fertile and sterile λhh plants is dependent on small amounts of ι plasmon which may or may not be transmitted by the *hirsutum* pollen.

Convincing evidence for the occurrence of plasmon segregation is found in CO_2 sensitivity in Drosophila (L'Héritier and Teissier, 1937, 1938b; L'Héritier and Sigot, 1944–45). This character may or may not be transmitted by the sperm, the percentage of transmission varying from culture to culture. This is what would be expected if cytoplasmic granules, for which no distribution mechanism is known, were to be distributed at random to different sperm cells. Since the sperm cells lose a large amount of their cytoplasm during spermiogenesis, it can be seen that some of the corpuscles may or may not remain in the rest of the cytoplasm.

Also, for the cytoplasmic factor necessary for the formation of the

"killer" substance in Paramecium, the evidence for its particulate nature is convincing (Sonneborn, 1945a and b, and 1946). In this case, Preer (1946) has even been able to estimate the number of κ particles in the cell, and their speed of reproduction.

2. *Plasmon and Plastids*

The fact that most of the cases of cytoplasmic effects have been found in plants, has given rise to the suspicion that these cases may be due to plastid inheritance. This is of course excluded for the cases from animals.

In considering this possibility, it must be kept in mind that in many plant species plastids are regularly transmitted by the pollen. Ruhland and Wetzel (1924) have demonstrated with the aid of fluorescence microscopy, that in *Lupinus luteus, Narcissus incomparabilis* and *Crocus vernus,* minute chlorophyll-containing chloroplasts can be observed in the pollen tubes. In other cases, especially in Oenothera, the transmission of plastids through pollen has been concluded from genetic evidence. In Epilobium, on the other hand, plastid transmission through the pollen seems to be very rare.

In Epilobium, effects described as "cytoplasmic" may therefore be actually due to differences in plastids. This possibility has been suggested by Lehmann (1941b), and the question has been left open by Brücher (1940b) and by Ross (1940). Michaelis states that he does not believe that the plastids are responsible for the cytoplasmic effects. A paper (referred to in Michaelis and von Dellingshausen, 1942) in which he proposes to discuss his arguments has not been available to the reviewer.

The basis for Schwemmle's arguments is formed by Renner's (1934, 1936, 1937) theory of hybrid variegation in Oenothera. Two species with normal green leaves may give rise to variegated hybrids. This is explained by Renner on the theory, that the plastids of the two species are qualitatively different. In *odorata/Berteriana* crosses this would mean, that *Berteriana* plastids develop normally in all genotypes, while *odorata* plastids become green only in vI and Bv, but in other combinations are more or less inhibited. A plant with a genome inhibiting *odorata* plastids will therefore be green in parts which contain *Berteriana* plastids, yellow or white in parts carrying *odorata* plastids. The amount of variegation found in any plant will depend on the initial amount of *Berteriana* and *odorata* plastids, the maternal plastids being larger in amount initially, and on the speed of propagation of the two types. In this respect, *odorata* plastids in unsuitable genic environment are at a disadvantage.

This theory makes it possible to distinguish whether any plant has *odorata* or *Berteriana* plastids, by crossing it back to *Oenothera Berteriana* and *odorata*. If, for example, a plant has *odorata* plastids, it can give

variegated progeny only if pollinated by *Berteriana* pollen, never if pollinated by *odorata* pollen, except for the rare occurrence of plastid mutations. This has been found in Haustein's (Schwemmle *et al*, 1938) experiments a reliable method for the determination of the plastid constitution of a plant. Recovery does not interfere with this method, since the pollen complex coming from original *Berteriana* and *odorata* is not recovered.

If, in a variegated plant, a sector or shoot of uniform color arises, the plastids are considered pure *Berteriana* if the sector is green, pure *odorata* if it is pale: *E.g.*, 1v from a cross ♀ *Berteriana* × ♂ *odorata* is frequently variegated. If in a plant of this type a pale sector arises, this sector is supposed to contain *odorata* plastids only. This has been demonstrated by pollinating flowers of this sector or of their offspring by selfing with pollen of *odorata* and of *Berteriana*. Only in the latter case can variegation be obtained. Since the cytoplasm is transmitted from the mother only, such plants would contain *Berteriana* cytoplasm and *odorata* plastids.

In this way, Schwemmle (1938) has succeeded in obtaining a number of genic complexes with *Berteriana* plastids in *odorata* cytoplasm which have given information concerning the relative rôle of plastids and cytoplasm in the maternal effects.

It has turned out, that the cytoplasm is not concerned with the inhibitions found in 1v and BI in *odorata* "cytoplasm," nor with any of the lethal effects. For instance, the $\mathring{o}\delta$BI is not inhibited with *Berteriana* plastids, as it is with *odorata* plastids, even though unrecovered gene complexes may be introduced. The $\mathring{o}\delta$II, which is lethal with *odorata* plastids, is perfectly viable with *Berteriana* plastids.

Also some of the matroclinous effects have turned out to be due to the plastids. This has been demonstrated for leaf shape in BI. $\beta\epsilon\rho$BI has broad, dentate leaves intermediate between *odorata* and *Berteriana*, while $\mathring{o}\delta$BI has ovate leaves with rather smooth margins, almost like *odorata*. This difference is maintained in the process of recovery. $\mathring{o}\delta$BI with *Berteriana* plastids has intermediate leaves, just as $\beta\epsilon\rho$BI. Schwemmle concludes that the *odorata* plastids in BI cause an inactivation of the B complex which carries the genes for broad, dentate leaves.

These experiments prove, that the plastids, in interaction with the genes, are able to exert definite morphogenetic effects. It is astonishing, that certain genotypes, like 1I, die as embryos under the influence of *odorata* plastids, since in the embryo the chloroplasts do not show any photosynthetic activity.

While these results give an experimental basis to the assumption, that "cytoplasmic" effects may be caused by the plastids, a non-plastid cytoplasmic effect could be established in the same series of experiments (Schwemmle *et al*, 1938). Hypanthium length and petal size decrease

gradually in the course of successive generations of selfing. But corresponding generations in *odorata* cytoplasm could be shown to have always longer hypanthia and larger petals than the same complex in *Berteriana* cytoplasm. This difference is due to the plasmon and not to the plastids. This has been established by comparing a large number of complexes, B1, vI, BI, 1I, BII and vII in *odorata* and in *Berteriana* cytoplasm, but always with *Berteriana* plastids. The *odorata* plasmon has the tendency to cause longer hypanthia and larger petals than *Berteriana* cytoplasm. A slight additive effect of the plastids on hypanthium length has been suggested by Schwemmle in a more recent publication (1941).

It must be concluded, that cytoplasmic effects may actually be caused by plastids, and that the differentiation between plasmon and plastids will have to be investigated in every case. It has been possible, however, to establish, that not all cytoplasmic effects are due to the plastids, and some must be attributed to a plasmon.

3. *Plasmon and Viruses*

It has also been suggested, that cytoplasmic effects may be due to viruses which are normally transmitted by the egg cell. This is possible in analogy to the transmission of bacterial and yeast symbionts by insect eggs, and to the well known transmission of spirochaete recurrentis from infected female ticks to their offspring and of plant viruses through seeds and pollen (Milbrath and Zeller, 1945; Reddick, 1931).

The characteristics by which a maternally transmitted virus disease can be distinguished from a plasmon action have been discussed by Darlington (1944). The virus theory must be accepted if it is possible to transmit the effect from organism to organism by means other than the egg, *i.e.* by infection with ground tissue or by transplantation experiments.

Experiments of this type have been performed by Rhoades (1933) with male sterile maize, by Ross (1941) with inhibited and non-inhibited hybrids in *hirsutum* Jena plasmon, and by Robertson (1937) in a case of maternally transmitted plastid abnormality in barley. The results of all these experiments have been negative. In CO_2-sensitive Drosophila, it has proved impossible to transmit the sensitivity to resistant animals by feeding or injecting of ground tissues from susceptible animals. But L'Héritier and de Scoeux (1946) report that they succeeded in transmitting CO_2-sensitivity to resistant ovaries by transplantation. Kalmus and Mitchison (1946), performing the same experiment, came however to the opposite result. The question, whether CO_2-sensitivity is due to a transmissible virus, must therefore be left open.

It may be argued, that even these cases in which transmission through transplantation is impossible, may be due to a virus which is unable to

leave the living cell, and can therefore only be transmitted through multiplying cells. But it seems, that in this case there would be no actual difference between a virus and a plasmon. Attributing cytoplasmic effects to a virus of this type would only postulate that the plasmon is particulate — a question which is left open by the use of the term plasmon — and that a plasmon difference may have arisen in evolution through infection with a virus. But many workers on viruses hold the opinion that viruses originate *de novo* intracellularly by changes of cytoplasmic constituents. If a virus arising in this way could be transmitted through the egg cells only, it would be identical (not indistinguishable!) with a change in plasmon.

A difference between most cases of virus disease, and of cytoplasmic effects, is the dependence of the latter on segregating genes. Genes are certainly involved in the susceptibility of different strains to virus infections. The para-crinkle virus in the King Edward potato is pathogenic for other strains, but not for the King Edward strain (Salaman and Le Pelley, 1930). The resistance of *Nicotiana glutinosa* to tobacco mosaic disease can be transferred to *N. tabacum*, by substituting one *glutinosa* chromosome for the corresponding *tabacum* chromosome (Gerstel, 1943, 1945). But the susceptibility to virus diseases does not seem to be dependent on a small number of segregating genes, such as the genes responsible for male sterility in flax, sugar beets, *Dactylis glomerata*, and Streptocarpus, and the deformans gene in *Epilobium hirsutum* Kew.

4. *Plasmon and Plasmagenes*

It has been suggested, especially by Lehmann (1939b), that cytoplasmic effects may be due to self-reproducing gene products. Self-reproducing cytoplasmic gene products have been assumed repeatedly in theories on the mechanism of gene action. Such particles have been called plasmagenes (Wright, 1941, 1945; Spiegelman, 1945, 1946) and cytogenes (Lindegren, 1945). The assumption has been made, that these particles are free cytoplasmic replicas of the gene. The cytogenes of Lindegren and the plasmagenes of Wright and of Spiegelman involve different assumptions as to their nature (see Spiegelman, 1946). For the present discussion it is sufficient, however, to designate by the term "plasmagene" any gene product which is carried in the cytoplasm and able to reproduce its own kind. It should be pointed out in this connection that Darlington (1944) uses the term "plasmagene" with a different meaning, designating by this word any self-reproducing cytoplasmic particles *i.e.* a corpusculate plasmon.

The existence of plasmagenes in the sense used here has been demonstrated beyond doubt in experiments by Lindegren and Spiegelman

(Lindegren, 1945; Lindegren *et al*, 1944; Spiegelman, 1945, 1946; Spiegelman and Lindegren, 1945a and b; Spiegelman *et al*, 1944, 1945) on yeasts. It has been indicated previously that the ability of yeast strains to break down certain sugars, galactose and melibiose, is dependent on one gene each, and on the presence of the sugars in the medium. Yeast cells containing these genes are able to form the enzymes galactase and melibiozymase in presence of the respective sugars. If strains containing these genes are crossed to strains which contain the corresponding alleles causing inability to form these enzymes, haploid yeast strains will be obtained which carry the "inactive" allele. If, however, the yeast has been kept on a medium containing these two sugars both before and after the crossing, even those strains containing the "inactive" allele will continue to form the enzymes, as long as the sugars are present in the medium. If the sugars are missing, both strains stop producing the enzymes. But strains containing the alleles necessary for the formation of the enzymes are able to form the enzymes anew, while strains containing the opposite alleles are not.

These results indicate, that two pairs of alleles determine whether a cell is able to start formation of galactase and melibiozymase in presence of the respective sugars. If, however, a cell does form enzymes, it can continue to do so indefinitely as long as the sugars are present in the medium. It appears, therefore, that either the enzymes themselves or a gene product necessary for the formation of the enzymes is able to continue itself, as long as the necessary environmental conditions exist.

Whether the cytoplasmic effects summarized in this review are due to plasmagenes or not has been the object of a violent discussion between Lehmann and Michaelis. Lehmann's position is founded on a number of experiments of the type described earlier in this paper for crosses of *Epilobium roseum* and *parviflorum*. Hybrids of two *roseum* strains which did not show any abnormalities were outcrossed to *E. parviflorum*. The progeny segregated into inhibited and uninhibited plants. It had to be concluded, that "inhibitory" genes cause abnormalities in *roseum-parviflorum* hybrids, and that the inhibitions caused by the genes from different roseum strains are of different strength. The plasmon influence is seen from a quantitative difference in the inhibitions of the hybrids in different *roseum* cytoplasm. Lehmann was first inclined to consider these cytoplasmic effects as caused by maternal transmission of gene controlled growth substances (1936b). But since Brücher (1938) and Michaelis (1938) demonstrated that these differences are transmitted unchanged through a number of generations of backcrossing, Lehmann (1939b) has modified his position in ascribing to the gene products the ability to reproduce them-

selves like viruses. This assumption would fit the definition of a plasmagene given above.

If this assumption is correct, then cytoplasmic effects in inter-strain or inter-specific crosses would tend to duplicate the genic effects, both in nature and in degree. This is certainly true in many cases of cytoplasmic effects. The light strain in Habrobracon (Kühn, 1927) and the Japanese strains in Lymantria (Goldschmidt, 1924) contain both genes for light pigmentation and a plasmon causing lighter pigmentation, as opposed to the darker strains. *Oenothera odorata* (Schwemmle *et al*, 1938) has genes causing both larger hypanthia and larger petals than *Oe. Berteriana*, and a plasmon acting in the same sense.

Lehmann believes that the same is true for different strains of *Epilobium hirsutum*, but Michaelis obtained different results. Michaelis (1938) used three different *E. hirsutum* strains, Jena, Munich, and I which gave different degrees of inhibition if outcrossed as females to *E. luteum*. If hybrids of two *hirsutum* strains were outcrossed as female parents to *luteum*, 1:1 segregations into more or less inhibited plants were found. These hybrids corresponded exactly to those obtained in crosses of the pure strains directly to *E. luteum*. The segregating "inhibitory" factors of the three *hirsutum* strains seem to be one series of multiple alleles linked to an anthocyan factor. In this way, the inhibitions due to a certain factor in the same plasmon can be compared. It is found that the relative degree of inhibition caused by the different genes in any cytoplasm is always the same, the Jena allele causing the slightest inhibition, while the Munich allele is most strongly inhibiting. If, on the other hand, the inhibiting influence of the different plasmons on the same genes is compared, the Jena plasmon is always most strongly inhibitory, while the Munich plasmon has the weakest influence.

It is concluded, that, since the Jena gene has the slightest inhibitory influence, it cannot be responsible for the strong inhibitory action of the Jena plasmon. The same applies to the other strains. While it is quite possible, that some of the cytoplasmic effects are due to plasmagenes, this seems unlikely for the inhibitions observed in Epilobium hybrids.

5. *Plasmon and Dauermodifications*

Dauermodifications have been defined above as cytoplasmic changes induced by an environmental factor which decrease in penetrance and expressivity in succeeding generations in the absence of the inducing stimulus. There has been some discussion as to whether dauermodifications and plasmon effects are essentially the same phenomenon or not. v. Wettstein (1937) especially insists in drawing a clear distinction between

these two phenomena, while Jollos (1939) and Knapp (1938) believe them to be fundamentally identical, apparent differences being due to differences in the method of demonstration. Michaelis (1933) takes an intermediate position by comparing the change of *luteum* plasmon under the influence of *hirsutum* genes to a dauermodification.

There seems to be no doubt, that in both phenomena cytoplasmic factors are involved which are able to reproduce themselves at least temporarily. The plasmon is usually more constant than dauermodifications, but in Honing's experiments in Nicotiana (1932), a decrease of plasmon action is obvious already in F_2. The dependence of plasmon action on the presence of plasmon-sensitive genes seems to be universal, while no influence of genes on dauermodification has yet been demonstrated.

In view of the facts described, and in the absence of any further experiences, it seems to be a matter of convenience, whether dauermodifiactions and plasmon effects should be considered as different degrees of the same phenomenon (Jollos, 1939, Knapp, 1938), or as fundamentally different effects. The reviewer is inclined to regard the second procedure as heuristically more valuable, since up to now the two phenomena are quite distinct, and no intermediate cases have been described. On the other hand, there seems to be no doubt that some kind of interaction of these two cytoplasmic effects in the production of the phenotype will occur.

Spiegelman (1946), in his discussion of Sonneborn's paper (1946), gives a model for possible interaction of plasmagenes as demonstrated by his experiments in yeast, and cytoplasmic factors of the Kappa type. It has been pointed out above, that the Kappa factor is a typical plasmon constituent, the gene *K* being inhibited by non-kappa cytoplasm. Preer (1946) gives evidence, that the origin of Kappa is not dependent on the *K* gene. Lindegren and Spiegelman's results, on the other hand, have similarities with dauermodifications, the phenotype "enzyme production" being initiated by an environmental factor, presence of the respective sugars, being transmitted through the cytoplasm, and gradually disappearing in the course of several hours in the absence of the initiating substrate. The genetic background decides whether the phenotype "enzyme production" can be produced at all.

It has been stated, that Lindegren and Spiegelman's results have been interpreted in terms of plasmagenes, while Sonneborn's results are best described as interactions between a particulate plasmon and a plasmon-sensitive gene. Spiegelman's interpretation assumes, that a plasmagene produced by a plasmon-sensitive gene reacts with a plasmon particle in producing the phenotypic effect. It is not clear, whether such an interpretation can be generalized to cover all dauermodifications and plasmon effects. At all events, the proposed theory assumes a difference and inter-

action between plasmagene and plasmon. It seems preferable to distinguish the two effects for the time being, keeping in mind, that they will probably turn out to be connected in some way.

IX. Plasmon and Evolution

1. *The Relation of Plasmon Differences and Taxonomic Differences*

An old theory, going back to Boveri (1918) and Jaques Loeb (1916), assumed that the fundamental differences between species, genera and higher taxonomic units are based on cytoplasmic differences, and that only the differences between varieties and strains are due to genes. This theory seemed at first to gain support from the findings of v. Wettstein (1926, 1928a) and of Skalinska (1928, 1929, 1930a), that the wider the taxonomic difference between two organisms was, the greater was the difference between reciprocal hybrids.

More recent investigations have tended to disprove this contention. Most of the cytoplasmic differences known now are found between subspecies, varieties and strains of the same species. The larger reciprocal differences observed in species and genus hybrids are explained by a higher degree of unbalance of the genome. Especially impressive have been the investigations of Michaelis who found that the plasmon differences between different local strains of *Epilobium hirsutum* are larger than those found between different species.

Michaelis (1942d) tested 222 different local strains of *E. hirsutum* for genes sensitive to Jena plasmon, by crossing them as pollen parents to Jena plants as females. They gave more or less inhibited hybrids, running through the whole series of inhibitions. As a general rule it was found, that strains of Central European origin contained less plasmon-sensitive genes than strains from the Atlantic regions of Europe. Some South African strains gave rise to the strongest inhibitions (Michaelis and von Dellingshausen, 1942).

Furthermore, 186 different *hirsutum* strains were crossed as female parents to pollen from a strain from Kew. Only seven of them yielded strongly inhibited hybrids, including the Jena strain. It may be of importance, that all these seven strains originated in Thuringia. Crosses between the seven inhibiting strains demonstrated that their plasmons were not identical.

These findings suggest that the plasmon of the species *Epilobium hirsutum* has undergone changes since the time the species has been established. Similar results on an interspecific level have been obtained by Oehlkers (1940) in Streptocarpus. The plasmon of *S. Rexii* tends to inhibit female fertility in hybrids with *S. Wendlandii*. The plasmons of

two other species of the same genus behave the same way. *S. Wendlandii* plasmon on the other hand, causes, in combination with certain *Rexii* genes, male sterility, and the transformation of stamens into pistils. The plasmon of *S. Comptoni* behaves the same way. But from a taxonomic point of view, *S. Comptoni* is much more different from most other species, since it shows a high degree of morphological differences, and gives progeny if crossed to *S. Wendlandii* only in rare instances and in one direction only (Oehlkers, 1941). Here the evolution of genomes, plasmons and crossing barriers has apparently proceeded with different speed in different species.

The experimental evidence suggests that the plasmon in general is not particularly stable. Both sudden changes resembling mutations and gradual changes of the type of the "dauermodifications" have been observed.

In many strains containing plasmon-dependent characters, individuals occur once in a while spontaneously which do not show the character and do not transmit it to their offspring. In the CO_2 sensitive strain of Drosophila, resistant individuals appear occasionally which transmit this resistance to their offspring (L'Héritier and Teissier, 1938b). Similarly, out of 49 pollen fertile plants from the pollen sterile maize strain, three gave only pollen fertile progeny (Rhoades, 1933).

Besides these spontaneous changes, plasmon changes under the influence of foreign genes and of environmental conditions have been observed. The best established case is the gradual change of the *E. luteum* plasmon under the influence of 18 generations in combination with a *hirsutum* genome (Michaelis, 1933; Michaelis and Wertz, 1935). A convincing case of plasmon change under environmental conditions has been described. Ross (1942b) observed, that uninhibited shoots arise in inhibited (♀ *hirsutum* × ♂ *parviflorum*) hybrids especially in fall and under short day conditions. These shoots transmit their uninhibited character to their offspring. Ross concludes, that short day conditions have irreversibly restored the balance between plasmon and genome.

Considering the difficulty of establishing plasmon differences, the material suggesting changes in plasmon seems sufficient to suggest, that the plasmon is not very stable. Plasmon changes seem to occur not infrequently in established species, and do not seem to form the basis for new taxonomic differences. This is especially plausible since a large number of the cytoplasmic characters known are not expressed phenotypically, except in the presence of unbalanced genomes.

2. *The Plasmon as a Crossing Barrier*

The main way which can be postulated in which the plasmon may affect evolution is by acting as a barrier for species crosses, and as a mecha-

nism of isolation. A peculiarity of this mechanism of isolation would be that it would sometimes prohibit certain genes or gene complexes from combining with the remaining genes in the foreign cytoplasm.

This has been demonstrated in crosses ♀ *Phaseolus vulgaris* × ♂ *Phaseolus multiflorus* by Lamprecht (1941, 1944). In this case the cytoplasmic basis is not completely clear, since the reciprocal cross has not been analysed. But the situation is so similar to that frequently found in cases of cytoplasmic inheritance that it may be taken as an example. The F_1 resembles phenotypically the father *multiflorus*, indicating that many *multiflorus* genes are dominant. The F_1 has a low fertility, over 90% of the gametes being killed. The F_2 obtained from the remaining gametes is phenotypically similar to vulgaris, indicating that a large number of *multiflorus* genes has been eliminated. From this F_2, different lines of hybrids could be established by selfing. They breed true, and contain different combinations of *vulgaris* and *multiflorus* genes. There are only two alleles from *multiflorus* which could not be established in these hybrids, one determining the position of the cotyledons, the other one affecting the shape of the stigma. Only rarely plants are found which contain either one or both of these genes in *vulgaris* cytoplasm, and if they are present, they are usually sterile or of reduced fertility. A remarkable fact is that progeny from the fertile plants carrying either one of these two *multiflorus* genes contain an unusual number of individuals carrying the corresponding allele from *vulgaris*. This occurs even, if the parental plant had been homozygous for the allele from *multiflorus*. Even allowing for a high mortality rate of gametes carrying the *multiflorus* allele, Lamprecht concludes that its mutation rate to the *vulgaris* allele in *vulgaris* cytoplasm must be at least 4%–6%.

Lamprecht interprets his results by complicated assumptions, involving "progenes" carried in the chromosomes which rebuild the genes at every meiosis with the aid of the cytoplasm. Such an hypothesis seems unnecessary in view of the close agreement of this case with other cases of cytoplasmic effects. The two *multiflorus* genes which cannot be established in the hybrid lines must be regarded as sensitive towards *vulgaris* plasmon. This plasmon-sensitivity is expressed by high gamete mortality, low zygote fertility and high mutation rate.

3. *The Influence of the Plasmon on Mutation Rate*

The only novel feature in the Phaseolus case is the high mutability of the plasmon-sensitive genes in foreign cytoplasm. Except for Schwemmle's genome changes described above (Schwemmle *et al,* 1938) in which an explanation other than mutation was considered preferable, there exists only an observation by Stubbe (1935) on mutation rates in different

plasmons. He compared the mutation rate of *Epilobium hirsutum* and of *E. hirsutum* in *luteum* plasmon from the 11th generation of backcrossing after irradiation with X-rays (3000*r*). He found 18 mutants amongst 865 tests in *hirsutum* (2.08 ± 0.48%), 30 mutants amongst 630 tests in λ*hh* (4.76 ± 0.84%). The possibility, that different mutation rates may be found in different cytoplasms can therefore not be excluded.

X. Conclusions

The material summarized in this review seems to establish the fact that transmission of cytoplasmic characters actually exists. They are in some cases due to plastids, in others probably due to plasmagenes and to viruses. In still other cases a general quality of the cytoplasm, such as a characteristic degree of hydration of the plasmon colloids, a particular degree of viscosity or osmotic value may be transmitted purely maternally. There is no reason anymore to doubt the existence of a plasmon, if the definition of the plasmon is made sufficiently broad to include all the possibilities mentioned above.

On the other hand, it has turned out in recent investigations, that the existence of a plasmon does not conflict with the well-founded views on gene behavior. It has been shown, that in determining phenotypic characters, the plasmon usually depends on the presence of certain genes. The theory of plasmon-sensitive genes offers a sound foundation for the interaction of plasmon and genes, indicating that the same genes act differently in different plasmons.

The accent in research on cytoplasmic inheritance has therefore shifted during the last ten years from attempts to establish the presence or absence of a plasmon to a study of gene effects in different plasmons. These studies have resulted in a promising start in the physiological interpretation of the interaction of genes and cytoplasm, and it seems that they might offer a way of attacking the problem of the primary action of the gene. Research on cytoplasmic inheritance has therefore proceeded from the study of an isolated phenomenon, unconnected with the bulk of genetic knowledge, to become a part of physiological genetics.

From an evolutionary point of view, the results obtained in the study of cytoplasmic inheritance are also consistent with the usual genetic theory. There are indications both from laboratory experiments and from taxonomic data, that the plasmon is at least not more stable than the genes, and cannot be held responsible for a different type of evolution. Its most important influence seems to be its ability to produce more or less effective crossing barriers which may affect single genes or gene groups, chromosomes or whole genomes. Differences in mutation rate in different plas-

mons have been suggested by some experiments, and if they can be more firmly established, they would be a factor to be considered in the study of evolution.

XI. REFERENCES

(Papers marked by an asterisk * have not been seen by the reviewer in the original.)

Altenburg, E., *Amer. Nat.* **80**, 559–567 (1946).
Anderson-Kottö, I., *Z.i.A.V.* **56**, 115–201 (1930).
Bateson, W. and Gairdner, A. E., *J. Genet.* **11**, 269–276 (1921).
Becker, G., *Z.i.A.V.* **60**, 17–38 (1932).
Bittner, J. J., *J. Hered.* **27**, 391–393 (1936a).
Science **84**, 162 (1936b).
J. Hered. **28**, 363–365 (1937).
J. nat. Cancer Inst. **1**, 155–168 (1940).
Science **93**, 527–528 (1941).
Canc. Res. **2**, 710–721 (1942a).
Science **95**, 462–463 (1942b).
Bittner, J. J., and Little, C. C., *J. Hered.* **28**, 117–121 (1927).
Boveri, Th., *Arch. EntwMech. Org.* **44**, 417–471 (1918).
* Breslavetz, L., *Zh. russk. bot. Obshch.* **15**, 149–162 (1930).
Brücher, H., *Z.i.A.V.* **75**, 298–340 (1938).
Z.i.A.V. **76**, 608 (1939).
Flora N. F. **34**, 215–228 (1940a).
Z.i.A.V. **77**, 455–487 (1940b).
Jb. wiss. Bot. **91**, 331–351 (1943).
Cadman, C. H., *J. Genet.* **44**, 33–52 (1942).
Caspari, E., *Arch. EntwMech. Org.* **130**, 253–281 (1933).
Z.i.A.V. **71**, 546–555 (1936).
Genetics **31**, 454–474 (1946).
Child, G. P., *Biol. Bull. Woods Hole* **77**, 432–442 (1939).
Chittenden, R. J., *J. Hered.* **18**, 337–343 (1927).
Bibliogr. genet. **3**, 355–439 (1927).
Chittenden, R. J., and Pellew, C., *Nature, Lond.* **119**, 10–11 (1927).
Clancy, C. W., and Beadle, G. W., *Biol. Bull. Woods Hole* **72**, 47–56 (1937).
Correns, C., *Bot. Zbl.* **84**, 97–113 (1900).
Ber. dtsch. bot. Ges. **36**, 686–701 (1908).
Z.i.A.V. **2**, 331–340 (1909a).
Z.i.A.V. **1**, 291–329 (1909b).
S. B. preuss. Akad. Wiss.* **20, 448–477 (1916).
Proc. Fifth Intern. Congr. Genetics **1**, 131–168 (1928).
Correns, C., edited by F. v. Wettstein, *Handb. Vererbungsw.* II H, 1–159, (1937).
Darlington, C. D., The Evolution of Genetic Systems, Cambridge 149 pp., (1939).
Nature, Lond., **154**, 164–169 (1944).
v. Dellingshausen, M., *Planta* **23**, 604–622 (1935).
Planta **25**, 282–301 (1936).
Demerec, M., *Proc. nat. Acad. Sci.* **20**, 354–359 (1934).
Proc. nat. Acad. Sci. **22**, 350–359 (1936).
Dickson, H., *Ann. Bot., Lond.* **3**, 113–129 (1939).
Dobzhansky, Th., *Proc. nat. Acad. Sci.* **21**, 443–446 (1935).

Dobzhansky, Th., and Schultz, J., *Proc. nat. Acad. Sci.* **17**, 513–518 (1931).
J. Genet. **28**, 349–386 (1933).
Dörries-Rüger, K., *Z.i.A.V.* **62**, 390–405 (1932).
East, E. M., *Amer. Nat.* **63**, 289–303, 402–439 (1934).
Genetics **17**, 175–202 (1932).
East, E. M., and Park, J. B., *Genetics* **2**, 505–609 (1917).
Fürtauer, R., *Jb. wiss. Bot.* **89**, 412–460 (1941).
Gairdner, A. E., *J. Genet.* **21**, 117–124 (1929).
* Gajewski, *Acta Soc. Bot. Polon.* **14**, 205–214 (1937).
Gerstel, D. U., *Genetics* **28**, 533–536 (1943).
Genetics **30**, 448–454 (1945).
Goldschmidt, R., *Arch. EntwMech. Org.* **101**, 92–336 (1924).
Arch. EntwMech. Org. **130**, 266–339 (1933a).
Arch. EntwMech. Org. **130**, 562–615 (1933b).
Scientia **53**, 94–99 (1933c).
Bibliogr. Genet. **11**, 1–185 (1933d).
Amer. Nat. **68**, 5–23 (1934).
J. Genet. **36**, 531–536 (1938).
Physiological Genetics, 375 pp., New York, McGraw-Hill, 1938.
Graze, H., and Schlenker, G., *Jb. wiss. Bot.* **82**, 687–695 (1936).
Haddow, A., *Nature, Lond.* **154**, 194–199 (1944).
Hadorn, E., *Verh. dtsch. zool. Ges.* **38**, 97–104 (1936).
Arch. Klaus-Stift. VererbForsch. **20**, Suppl., 82–95 (1945a).
Rev. suisse Zool. **47**, 167–176 (1945b).
Hämmerling, J., *Handb. Vererbgsw.* I,E, 1–69 (1929).
Harder, R., *Z. Bot.* **19**, 337–407 (1927).
Haustein, E., *Z.i.A.V.* **76**, 411–421 (1939a).
Z.i.A.V. **76**, 487–511 (1939b).
L'Héritier, Ph., and Teissier, G., *C. R. Acad. Sci., Paris* **205**, 1099–1101 (1937).
C. R. Acad. Sci., Paris **206**, 1193–1195 (1938a).
C. R. Acad. Sci., Paris **206**, 1683–1685 (1938b).
* *Publ. Lab. l'Ecole Norm. Sup. Biol. I*, 35–76.
L'Héritier, Ph., and Hugon de Scoeux, P., *Nature, Lond.* **157**, 729 (1946).
* L'Héritier, Ph., and Sigot, A., *C. R. Soc. Phys. Biol. Fr.* **72**, 108–110; **73**, 119–122 (1944–45).
Hill, J. B., *Bot. Gaz.* **80**, 84–92 (1925).
Bot. Gaz. **87**, 548–555 (1929).
Hinderer, G., *Jb. wiss. Bot.* **82**, 669–686 (1936).
Hofmann, F. W., *Genetics* **12**, 284–294 (1927a).
J. Agric. Res. **34**, 673–676 (1927b).
Honing, J. A., *Genetica* **12**, 441–468 (1930).
Meded. LandbHoogesch. Wageningen* **35, 1–12 (1931).
Z.i.A.V. **62**, 93–95 (1932).
Imai, Y., and Moriwaki, D., *J. Genet.* **33**, 163–168 (1936).
Johnson, T., and Newton, M., *Canad. J. Res.* **18**, 54–67 (1940).
Johnson, T., Newton, M., and Brown, A. M., *Sci. Agric.* **14**, 360–373 (1934).
Jollos, V., *Biol. Zbl.* **33**, 222–236 (1913).
Z.i.A.V. **12**, 14–35 (1914).
Arch. Protistenk. **43**, 1–222 (1921).

Jollos, V. (*continued*)
Z.i.A.V. **62,** 15–23 (1932).
Handb. Vererbw. ID, 1–106 (1939).
Kalmus, H., and Mitchison, A. N., *Nature, Lond.* **157,** 230 (1946).
*Kisch, E., *Z. Bot.* **36,** 513–537 (1941).
Knapp, E., *Biol. Zbl.* **58,** 411–425 (1938).
Koehler, K., *Z.i.A.V.* **94,** 243–325 (1929).
Kühn, A., *Nachr. Ges. Wiss. Göttingen*, 407–421 (1927).
Z. Zücht. B. **12,** 433–449 (1937).
Lamprecht, H., *Hereditas, Lund* **27,** 51–175 (1941).
Agrihortique Genetica **2,** 75–142 (1944).
Lehmann, E., *Z. Bot.* **10,** 497–511 (1918).
Bibliogr. Genet. **1,** 363–418 (1925).
* *Tübing. naturw. Abh.* **11,** (1928).
Z. Pflanzenz. **17,** 157–172 (1931).
Proc. Sixth Intern. Bot. Congr. Amsterdam **2,** 60–62 (1936a).
Jb. wiss. Bot. **82,** 657–668 (1936b).
Z.i.A.V. **76,** 602–607 (1939a).
Jb. wiss. Bot. **87,** 625–641 (1939b).
Jb. wiss. Bot. **88,** 284–343 (1939c).
Jb. wiss. Bot. **89,** 637–686 (1941a).
Jb. wiss. Bot. **89,** 687–753 and **90,** 51–98 (1941b).
Lehmann, E., and Schnitzler, O., *Ber. dtsch. bot. Ges.* **50,** 185–187 (1932).
Lehmann, E., and Schwemmle, J., *Bibl. bot., Stuttgart* **95,** 1–156 (1927).
Lewis, D., *New Phytol.* **40,** 56–63 (1941).
Lindegren, C. C., *Ann. Mo. bot. Gdn.* **32,** 107–123 (1945).
Proc. nat. Acad. Sci. **32,** 68–70 (1946).
Lindegren, C. C., Spiegelman, S., and Lindegren, G., *Proc. nat. Acad. Sci.* **30,** 346–352 (1944).
Little, C. C., *Science* **78,** 465–466 (1933).
Loeb, J., The Organism as a Whole, pp. 379, New York, 1916.
Mampell, K., *Genetics* **30,** 496–505 (1945).
Marshak, A., *J. Exp. Zool.* **72,** 497–510 (1936).
Doctor's Thesis, Harvard Univ., 1934.
Mather, K., *J. Genet.* **45,** 215–235 (1943).
Michaelis, P., *Biol. Zbl.* **49,** 302–316 (1929).
Biol. Zbl. **51,** 124–134 (1931a).
Ber. dtsch. bot. Ges. **49,** 96–104 (1931b).
Z.i.A.V. **62,** 95–102 (1932).
Z.i.A.V. **65,** 1–71, 353–411 (1933).
Züchter **6,** 303–306 (1934).
Planta **23,** 486–500 (1935a).
Ber. dtsch. bot. Ges. **53,** 143–150 (1935b).
Züchter **7,** 74–77 (1935c).
Protoplasma **27,** 284–289 (1937).
Z.i.A.V. **74,** 435–459 (1938).
Jb. wiss. Bot. **88,** 69–88 (1939).
Z.i.A.V. **77,** 548–567 (1940a).
Z.i.A.V. **78,** 187–222 (1940b).

Michaelis, P. *(continued)*
Z.i.A.V. **78,** 223–237 (1940c).
Z.i.A.V. **78,** 295–337 (1940d).
Z.i.A.V. **80,** 373–428 (1942a).
Z.i.A.V. **80,** 429–453 (1942b).
Z.i.A.V. **80,** 454–499 (1942c).
Biol. Zbl.* **62, 170–186 (1942d).
Michaelis, P., and v. Dellingshausen, M., *Jb. wiss. Bot.* **82,** 45–64 (1935).
Michaelis, P., and v. Dellingshausen, M., *Z.i.A.V.* **80,** 373–428 (1942).
Michaelis, P., and Wertz, E., *Z.i.A.V.* **70,** 138–157 (1935).
Milbrath, J. A., and Zeller, S. M., *Science* **101,** 114–115 (1945).
Murray, W. S., and Little, C. C., *Genetics* **20,** 466–496 (1935).
Myers, W. M., *Rec. Gen. Soc. Amer.* **14,** 55–56 (1946).
*Newton, M., and Johnson, T., *Bull. Dep. Agric. Can.* **160,** N.S., (1932).
Oehlkers, F., *Z. Bot.* **32,** 305–393 (1938).
Ber. dtsch. bot. Ges. **58,** 76–91 (1940).
Z. Bot. **37,** 158–182 (1941).
Biol. Zbl. **62,** 280–289 (1942).
* D'Oliveira, B., *Ann. appl. Biol.* **26,** 56–82 (1939).
Owen, F. V., *Amer. J. Bot.* **29,** 692 (1942).
J. Agric. Res. **71,** 423–440 (1945).
Pellew, C., *Biol. Rev.* **4,** 209–217 (1929).
* Piza, S. de Toledo, *Rev. Agric., S. Paulo* **21,** 32–39 (1946).
Plough, H. H., and Ives, P. T., *Genetics* **20,** 42–69 (1935).
Preer, J. R., *Proc. nat. Acad. Sci.* **32,** 247–253 (1946).
* Reddick, D., *2ème Congr. Intern. Path. Comp. Paris* **1,** 363 (1931).
Renner, O., *Biol. Zbl.* **44,** 309–336 (1924).
Ber. Math. Phys. Kl., Sächs. Akad. Wiss. Leipzig* **86, 241 (1934).
Flora **130,** 218–290 (1936).
Cytologia, Fujii Jubil. Vol., Tokyo 644–653 (1937).
Flora **136,** 117–214 (1942).
Renner, O., and Kupper, W., *Ber dtsch. bot. Ges.* **39,** 201–206 (1921).
Rhoades, M. M., *J. Genet.* **27,** 71–93 (1933).
Robertson, D. W., *Genetics* **22,** 104–113 (1937).
Ronne, H., *Acta ophthal.* **22,** 203–213 (1937).
Ross, H., *Ber. dtsch. bot. Ges.* **57,** 114–127 (1940).
Z.i.A.V. **79,** 503–529 (1941).
Planta **32,** 447–489 (1942a).
Naturwissenschaften **30,** 492–493 (1942b).
Planta **33,** 161–184 (1942).
Rudloff, K. F., *Z.i.A.V.* **52,** 191–225 (1929).
Ruhland, W., and Wetzel, K., *Ber. dtsch. bot. Ges.* **42,** 3–14 (1924).
Salaman, R. N., and LePelley, R. H., *Proc. roy. Soc. B.* **106,** 140–175 (1930).
Sansome, F. W., *J. Genet.* **33,** 359–364 (1936).
Saunders, E. R., *Proc. Fifth Intern. Congr. Bot. Cambridge* 202–203 (1930).
'Schlösser, L. A., *Z.i.A.V.* **69,** 159–192 (1935).
Schmidt, M., *Züchter* **4,** 191–198 (1932).
Schwanitz, F., *Z.i.A.V.* **62,** 232–248 (1932).
Schwemmle, J., *Z.i.A.V.* **34,** 145–185 (1924).
Jb. wiss. Bot. **67,** 849–876 (1928).

Schwemmle, J. (*continued*)
Z. Bot. **25,** 189–194 (1932).
Z.i.A.V. **79,** 171–187 (1940).
Z.i.A.V. **79,** 321–335 (1941).
Schwemmle, J., Haustein, E., Sturm, J., and Binder, M., *Z.i.A.V.* **75,** 358–800 (1938).
Schwemmle, J., and Zintl, M., *Z.i.A.V.* **76,** 353–410 (1939).
Sirks, M. J., *Proc. Kon. Akad. Wetensch. Amsterdam* **32,** 1066–1084 (1929).
Proc. Kon. Akad. Wetensch. Amsterdam **34,** 1057–1062 (1931a).
Proc. Kon. Akad. Wetensch. Amsterdam **34,** 1164–1172 (1931b).
Proc. Kon. Akad. Wetensch. Amsterdam **34,** 1340–1346 (1931c).
Genetica **13,** 209–631 (1931d).
Z.i.A.V. **73,** 367–373 (1937).
Genetica **20,** 121–158 (1938a).
Bot. Rev. **4,** 113–131 (1938b).
Skalinska, M., *Acta Soc. Bot. Polon.* **5,** 141–173 (1928).
Acta Soc. Bot. Polon. **6,** 138–164 (1929).
Acta Biol. exp. Varsovie **5,** 1–18 (1930a).
Proc. Fifth Intern. Congr. Bot. Cambridge, 250 (1930b).
Proc. Fifth Intern. Congr. Gen. **2,** 1343–1372 (1938).
Sonneborn, T. M., *Proc. nat. Acad. Sci.* **29,** 329–338 (1943a).
Proc. nat. Acad. Sci. **29,** 338–343 (1943b).
Proc. Ind. Acad. Sci. **52,** 90–91 (1943c).
Amer. Nat. **79,** 318–339 (1945a).
Ann. Mo. bot. Gdn. **32,** 213–221 (1945b).
Cold Spr. Harb. Symp. Quant. Biol. **11,** 236-248 (1946).
Spiegelman, S., *Ann. Mo. bot Gdn.* **32,** 139–163 (1945).
Cold Spr. Harb. Symp. Quant. Biol. **11,** 256-274 (1946).
Spiegelman, S., and Lindegren, C. C., *Ann. Mo. bot. Gdn.* **31,** 219–233 (1945a).
Spiegelman, S., and Lindegren, C. C., *J. Bact.* **49,** 256–269 (1945b).
Spiegelman, S., Lindegren, C. C., and Hedgecock, L., *Proc. nat. Acad. Sci.* **30,** 13–23 (1944).
Spiegelman, S., Lindegren, C. C., and Lindegren, G., *Proc. nat. Acad. Sci.* **31,** 95–102 (1945).
Stern, C., *Z.i.A.V.* **64,** 169–172 (1932).
Growth, Supplement 1940, 19–36, (1940).
Genetics **28,** 441–475 (1943).
Stern, C., and Heidenthal, G., *Proc. nat. Acad. Sci.* **30,** 197–205 (1944).
Stern, C., and Schaeffer, E. W., *Proc. nat. Acad. Sci.* **29,** 361–367 (1943a).
Stern, C., and Schaeffer, E. W., *Proc. nat. Acad. Sci.* **29,** 351–361 (1943b).
Stern, C., Schaeffer, E. W., and Spencer, W. P., *Amer. Nat.* **78,** 183–188 (1944).
Stubbe, H., *Z.i.A.V.* **70,** 161–169 (1935).
Sturtevant, A. H., *Publ. Carneg. Instn.* **399,** 1–62 (1929).
Tobler, M., *Z.i.A.V.* **60,** 39–62 (1932).
Tschermak-Seysenegg, A., *Züchter* **7,** 187–192 (1935).
Villerts, A., *J. Genet.* **43,** 223–235 (1942).
Weidner-Rauh, E., *Z.i.A.V.* **76,** 422–486 (1939).
v. Wettstein, F., *Z.i.A.V.* **33,** 1–236 (1924).
Nachr. Ges. Wiss. Göttingen 1926, 250–281 (1926).
Bibliogr. Genet. **10,** 1–216 (1928a).
Ber. dtsch. bot. Ges. **46,** 32–49 (1928b).

v. Wettstein (*continued*)
Nachr. Ges. Wiss. Göttingen 1930, 109–118 (1930).
Wiss. Woche Frankfurt a.M. **1,** 31–36 (1935).
Z.i.A.V. **73,** 349–366 (1937).
Winge, Ö., and Laustsen, O., *C. R. Lab. Carlsberg* **23,** 17–39 (1940).
Winkler, H., *Z.i.A.V.* **33,** 238–253 (1924).
Wright, S., *Physiol. Rev.* **21,** 487–527 (1941).
Amer. Nat. **79,** 289–303 (1945).
Zündorf, W., *Z.i.A.V.* **77,** 533–547 (1939a).
Z.i.A.V. **77,** 195–238 (1939b).

Genetics of Human Populations

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CONTENTS

Both heredity and environment determine what characters an individual will have. If, however, the genes deviate too much from the normal, no individual can come into existence. The same is true if the environment varies too much. Both heredity and environment can vary within the limits set by death, giving rise to individuals of very different types.

In experimental genetics the environment can be regulated, and this may be the reason why less interest has been attached to properties which are to some extent contingent on environmental factors. The primary point of departure in experimental genetics is to study which hereditary factors determine certain characters. The problems are by no means simple when human genetics is in question. In view of this I have suggested that characters of three types should be distinguished here, namely: 1) *hereditary* characters in the limited sense; 2) characters due to *environment;* 3) characters depending both on heredity and environment, and which I call *constellational* (Dahlberg, 1939). There is also the fact that characters may emerge at different ages. When making special investigations, the influence of age on the results can be eliminated by

restriction to definite age groups. When an entire population is in question, however, it is often necessary to take all its constituent age-groups into account. In that case, appropriate statistical methods have to be used. A distinction can also be made between characters which are of short duration (more or less accidental) and characters of longer duration (more or less permanent) in the above-mentioned three types.

The nature of the population determines to some extent the type under which a character is to be classified. To illustrate this, we will discuss a character normally contingent on both heredity and environment, that is to say a constellational character, where the hereditary factors necessary for its occurrence are found only in part of the population, and where the same is true of the environmental factor or factors which are similarly necessary. In a population where the necessary environmental factor is present in practically all individuals, the character will be determined by heredity; conversely, in a population where the hereditary factor is present in practically everyone, the character will be determined by environment. To take an example: paralytic dementia is usually due both to infection with syphilis and to the presence of hereditary tendencies such that infection with syphilis is followed in due course by a certain injury to the brain. In a population where everyone is infected with syphilis — as is said to be the case in Mongolia, for example — paralytic dementia will be a hereditary character, while the same character in populations where syphilis is less common must be classified as a constellational character.[1] In other words, the nature of such a character depends on the frequency of the environmental and the hereditary factors bearing on its occurrence (fig. 1.). Obviously, the dividing-lines between the groups of characters we have discussed are to a certain extent arbitrary, but the division should serve for practical purposes. In the following pages we shall only be discussing genes and characters that are hereditary in the limited sense of the word.

From a practical viewpoint, two main questions can be applied in human genetics. 1) What will the children of any given marriage be like? We can assume here that we have a more or less extensive knowledge of the two parents and of the characters of their relatives. 2) What will be the future make-up of a population, given more or less detailed data on its present make-up and on the crossings taking place within it?

It is clear that the latter question is a very important one in human genetics, and it is therefore surprising that it has not been more extensively studied. No empirical investigations along avenues of approach of the

[1] It has been asserted that paralytic dementia is due to infection with a certain kind of virus (syphilis à virus nerveux). As far as I can see, this theory is hardly tenable. In any case, we disregard this possibility in the above argument.

kind mentioned above have as yet been made. The problems have been treated solely theoretically. Studies of this sort are to be found in the most varied periodicals. It is our intention here to give a short survey of the problems from a theoretical point of view, since it may be presumed

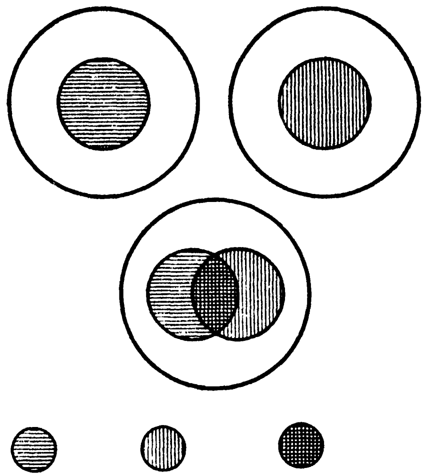


Fig. 1

Characters of different kinds. Above, to the right: a hereditary character; to the left: an environmental character. Below: a constellational character.

that human genetics will be directed more consistently along this avenue of approach in the future.

I. Populations in Panmixia

If crossings take place at random in the population, and if individuals of different heredity have the same chance of procreation, then we say that panmixia or amphimixis obtains. In this situation the population will change character at random. If the population is at all large, we can disregard this possibility of change and consider said population to be constant from generation to generation. If monohybrid heredity is in question, a knowledge of the frequency of the recessive and thus also of the corresponding dominant character enables us to calculate the frequency of the heterozygotes. The composition of the population is determined by the expression

$$r^2 + 2rd + d^2 \qquad (1)$$

where r is the frequency of the recessive gene, which we call *R*, and d is the frequency of the dominant gene, which we call *D* (fig. 2 and 3).

An interesting point is that the relation between heterozygotes, *RD*, and recessive homozygotes, *RR*, will be

$$\frac{RD}{RR} = \frac{2\text{rd}}{\text{r}^2} = \frac{2(1 - \text{r})}{\text{r}} \qquad (2)$$

The expression shows that if r is a very small number — *i.e.* if those having the recessive character are very few — then the heterozygotes will be proportionately very common. That is to say, the expression approaches ∞ when r approaches 0.

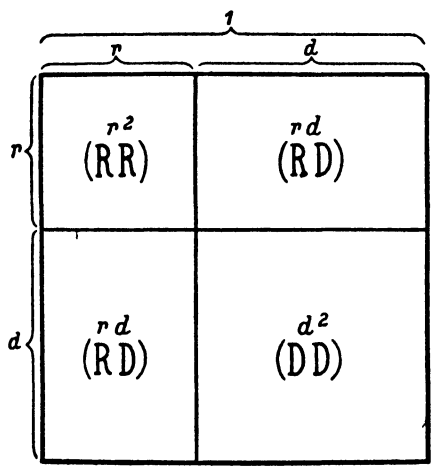


FIG. 2
Distribution of the zygotes (*RR*, *RD*, and *DD*) in a population in panmixia when the gene proportion r:d = 2:3.

Even if panmixia can usually be assumed to be present in many populations, there may be deviations which are of importance particularly when a rather longer view is taken. These deviations are:

1. Mutations
2. Selection
3. Assortative mating
4. Intermarriage
5. Effect of isolates

If a person gets married and has children at random, his genes may become changed so that the children show other hereditary factors than would otherwise have been expected (mutation). However, he may refrain from marriage and children (selection). Further, he may marry a person with a certain character (assortative mating), marry a relation (inter-

marriage), or marry within a limited circle (effect of isolates). All the conceivable deviations from panmixia in human populations come under these heads, and we shall now discuss them briefly.

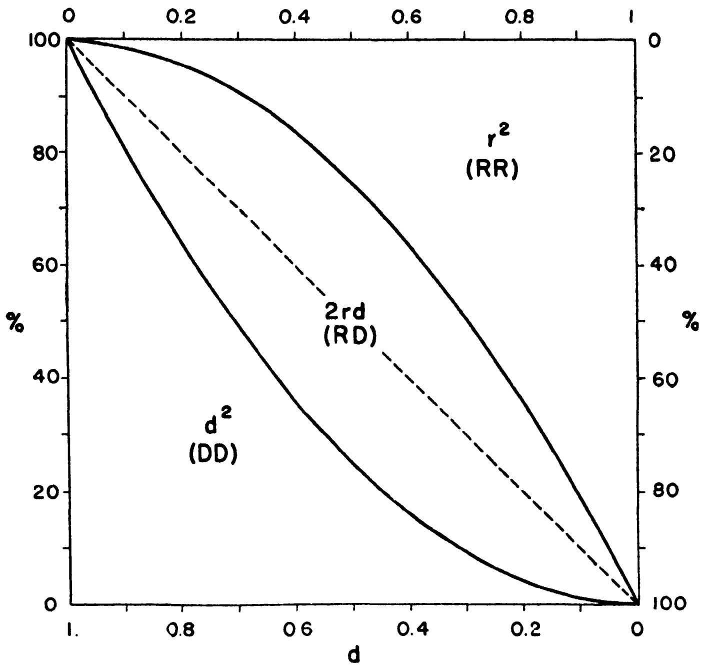


FIG. 3
Distribution of the zygotes in a population in panmixia at decreasing frequency of a recessive gene. *Cf.* formula 1.

The first to point out that the heredity of a population is of a constant nature in panmixia was Hardy (1908). However, Pearson (1903) had shown the same thing earlier for a special case, namely a population with a monohybrid gene frequency = 0.5. A survey of the composition of a population in panmixia was given by Dahlberg and Hultkrantz (1927).

II. MUTATIONS

As mutations are comparatively rare, they play little part in human populations when studied over rather shorter periods. We know little of the mutation frequency in man. In Sweden four or five individuals who suffer from juvenile amaurotic idiocy are born annually (Sjögren, 1931). They always die before maturity and never have children. Consequently, the genes must arise in an equal number through mutation. The rate of mutation therefore should be 1 :25000. For a special form of dwarfism

(chondrodystrophic dwarfs) E. T. Mørch (1941) has found a rate of mutation of 1:10000—12000. Haldane (1935) computed a rate of mutation of 1:50000 for hemophilia. Andreassen (1943) arrived at a figure of 1:53000 for the same disease.

In any case, it can doubtless be assumed that mutations are far too rare to produce noticeable differences between near generations of a nation. Taking a longer view of the problems, however, and particularly from the viewpoint of evolution, the mutations cannot but play a very large part. The mathematical questions in this field have been handled primarily by J. B. S. Haldane, R. A. Fisher, Sewall Wright, and others. But they fall outside the scope of this study, which merely intends a short-term treatment of the conditions in human populations.

III. Selection

Haldane (1924) has also submitted formulae for selection — that is to say the effect that results from inhibiting or preventing the propagation of character-bearers of a certain type. Now, if their propagation is prevented, this means that that of the other types will be favoured. It is customary only to think of the negative side when talking of selection, but obviously every selection has a positive side also.

If the gene is inherited dominantly and total selection is in question — *i.e.* the person with the character is completely prevented from propagating — the character will immediately be exterminated. If, on the other hand, recessive genes are in question, total selection does not result in an instant extermination, since the gene is to a certain extent recruited from heterozygotes; instead, there will only be a larger or smaller lowering of the frequency of the character in the subsequent generation. If the recessive gene has the frequency r, and the character thus has the frequency r^2, then after n generations the frequency of the persons with the character, r_n^2, will be determined by the following formula:

$$r_n^2 = \frac{r^2}{[1 + (n - 1) \cdot r]^2} \tag{3}$$

With the help of this formula, which has another form from that given by Haldane, and which has been taken from a work by Dahlberg-Hultkrantz (1927), we can calculate the make-up of the population with different initial frequencies of the gene (fig. 4).

It is seen that if a character is common, selection is very effective. If the character has the frequency of 25%, it falls in one generation to 11.1%. Gradually, however, selection becomes less effective, when the character becomes more and more rare. When a frequency of, say, 0.1% is reached, it will take ten generations before it is reduced by not quite

half, namely to 0.06%. This is because when a recessive character is rare, the heterozygotes are much more common for the gene than the homozygotes (see above).

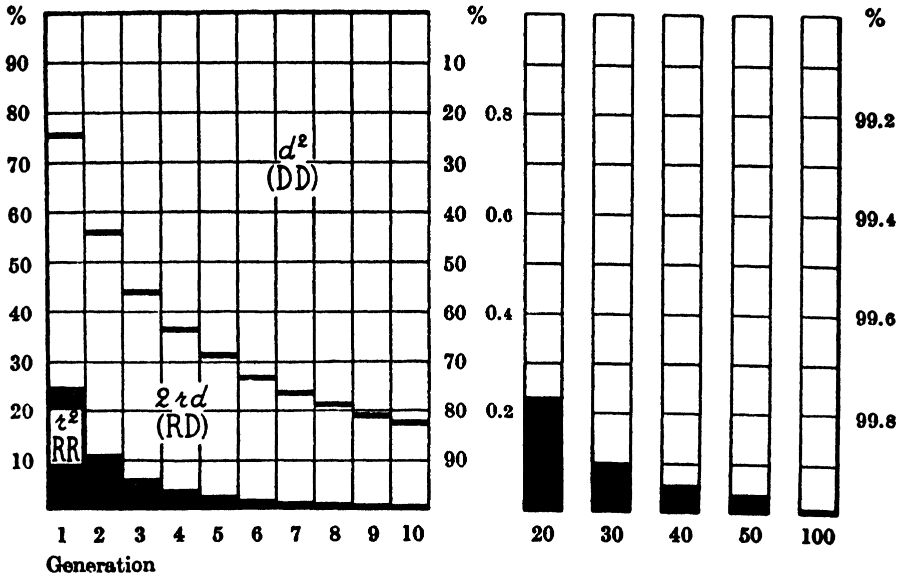


FIG. 4

Decreasing frequency of a recessive character in successive generations in total negative selection. *Cf.* formula 3.

It may be mentioned that the above equation can also be used to calculate the frequency of dominant characters. In monohybrid heredity we know the frequency of dominant characters when we know that of the corresponding recessive characters. It is more important to state that a favouring of the propagation of certain characters means, relatively speaking, that the ones not having the character are inhibited. In eugenics it is customary to discuss two kinds of measures, *i.e.* negative measures which will prevent the propagation of certain inferior types, and also measures of a positive kind, favouring the propagation of certain types. However, the effect of positive measures can be calculated with the help of the same formula as for partial negative selection.

If, then, we raise or diminish the propagation of persons with the recessive character by a certain proportion, k, of what it is in panmixia, the process will be determined by the following recursion formula (Dahlberg, 1946):

$$a_{n+1} = \frac{(a_n + b_n)^2 + k \cdot a_n(a_n + 2b_n)}{1 + k \cdot a_n(1 + 2b_n + c_n)} \qquad (4)$$

In this formula a_{n+1} denotes the frequency of the recessive homozygotes in the generation $(n + 1)$ and a_n their frequency in the previous generation, $2b_n$ is the frequency of the heterozygotes[2], and c_n the frequency of the dominant homozygotes in this generation. If $k = 0$, this means that the propagation is not being changed from that in panmixia. Nor is there then any change according to the above equation. If k is a negative number < 1, there is a decrease in the frequency of the recessive character. If $k = -1$, then total negative selection obtains. The formula is then simplified to agree with formula 1 above. If k is a positive number, this means that the propagation of the recessive characters is favoured.

The corresponding recursion formula for the heterozygotes is:

$$2b_{n+1} = \frac{2(k \cdot a_n + a_n + b_n)\,(b_n + c_n)}{1 + k \cdot a_n\,(1 + 2b_n + c_n)} \qquad (5)$$

With the help of formulae 4 and 5 the frequency of dominant homozygotes can be computed.

If the propagation of the recessive is inhibited, it means the favouring of that of the dominant, though only of those who marry persons with the dominant characters. If all the persons with the dominant characters are to be influenced, even those marrying recessive homozygotes, or — which is the same thing — if only recessive homozygotes marrying one another are to be influenced, then the process will be according to the following recursion formula (Dahlberg, 1947), where the symbols have the same meaning as before:

$$a_{n+1} = \frac{(a_n + b_n)^2 + k \cdot a_n^2}{1 + k \cdot a_n^2} \qquad (6)$$

The recursion relation for the heterozygotes is the following:

$$2b_{n+1} = \frac{2(a_n + b_n)\,(b_n + c_n)}{1 + k \cdot a_n^2} \qquad (7)$$

When this is the situation, then, propagation is favoured or inhibited in all marriages to which dominant character-bearers are a party. The frequency of the dominant homozygotes is obtained from the above formulae by subtracting the recessive homozygotes and the heterozygotes from the total number.

As stated above, measures aimed at *inhibiting* the propagation of a recessive character are found to have very little effect if the character has

[2] Haldane has given a formula for partial negative selection, valid for aquatic organisms "which shed their gametes into water." This formula is correct for selection in human populations caused by differential mortality in younger years but not for selection due to differential fertility. However, the difference between the figures obtained with the help of Haldane's formula and the one given here are rather small.

a low frequency. If its frequency is high, the effect will be more noticeable provided the amount by which propagation is checked is not unduly small.

In the case of partial negative selection where the reproduction rate is half that of the rest of the population (*i.e.* k = ½), the frequency will have fallen from 0.1% to 0.075% after 10 generations (fig. 5). The effect of

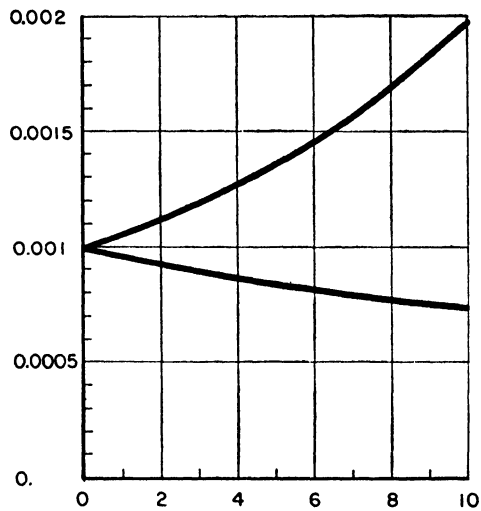


Fig. 5

Frequency of persons with a recessive character in successive generations at partial negative selection when their procreation is half (k = −½; *cf.* formula 4) that of the corresponding dominant character (the lower curve), and at partial positive selection when their procreation is double (k = 1) that of persons with the corresponding dominant character (the upper curve).

negative selection is, in other words, insignificant. If equally strong negative selection works against a dominant character with a frequency of 0.1%, the effect will be that the frequency of the character will be reduced to 0.0001% (fig. 6). Here, in other words, the effect is very much greater.

Positive selection, on the other hand, has a comparatively strong effect which in some cases is rather astonishing. If a recessive character has a frequency of 0.1% and the reproduction of all the individuals is double that of the rest of the population, the character will have risen only to nearly 0.2% after 10 generations, but in the case of all the dominant characters being favoured to the same extent, we get, after an equal number of generations, a rise to 34% (fig. 7).

If, finally, a character determined by two or more factors is in question, it is easy to show that the process is slower than in monohybrid recessivity with the corresponding frequency of the said character. That is to say,

there are proportionately fewer of the genes present in the homozygotes in recessive polyhybridism than in monohybridism, so that the greatest effect from selection is obtained with an ordinary monohybrid character. From a practical point of view it is important that the selection has so small an effect on rare characters in human populations. For this reason no great effect can be expected from negative eugenic measures, such as

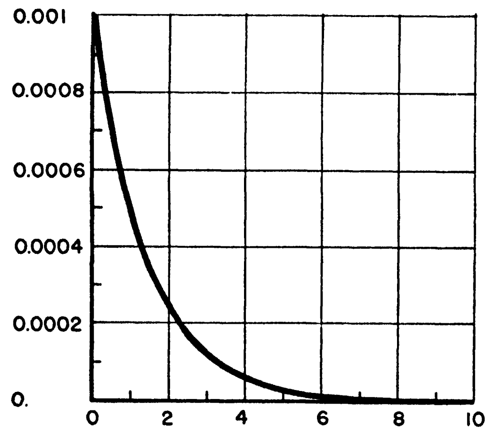


Fig. 6

Frequency of persons with a dominant character at partial negative selection when their procreation is half (k = 1; *cf.* formulæ 6 and 7) that of the corresponding recessive character. The curve happens to be the same as the one which is obtained at total negative selection for a sex-linked character. *Cf.* formula 9.

sterilisation. It must also be remembered that a "character" against which such action is taken, as a rule, actually consists of several different hereditary characters. Thus, the imbecile, the blind, etc. are built up of different hereditary types of imbecility and blindness, and also of characters of the kind due to environment. The effect of selection on such groups of individuals will naturally be far weaker than if the group had been homogeneous and consisted of a single character.

Practically speaking, continued negative selection must lead to elimination of the character and the gene against which it is directed. There is, however, the contingency that the gene and the character may arise by mutation. A state of balance will therefore gradually come to be established between mutations and selection. The author has given this limit at which selection ceases to have effect the name of *the least heterozygote frequency*. If this is reached, the mutation frequency can be computed by investigating how many persons with a character in each generation are prevented from propagating, since the mutation frequency

corresponds to the number of genes that are eliminated. If a gene arises only once through mutation it must reach a certain frequency — *i.e.* appear in a certain number of heterozygotes — in order to occur in homozygote form via intermarriage. The frequency of the gene can never be pushed below this limit.

In recessive sex-linked heredity, selection will act on the male sex as in dominance, and on the female as in recessivity. When selection has lowered the frequency of the character, so that in practice all the women can be accounted heterozygotes, the frequency of the character will, with total selection, be diminished by half in every generation. Hemophilia is an example of this. Sweden contains about 100 individuals suffering from this disease, all of them males. If all these are prevented from propagating, the character will be maintained by the number of heterozygotes who are present among the women, and who number the same. Provided they are of average fertility and that the population is constant from generation to generation, the number of hemophilics in the next generation will be 50. That is to say, the 100 female heterozygotes have twice their own number of offspring. Half of the children are boys, and half of them in their turn will suffer from the disease. The general formula for selection in monohybrid sex-linked heredity, when the gene frequency is r, may be written in the following manner:

$$r_n = \frac{r}{2^n-(2^n-1)r} \tag{8}$$

When the character is rare, the formula can be simplified approximately to:

$$r_n = \frac{r}{2^{n-1}} \tag{9}$$

The character is then eradicated in a few generations (Sköld, 1944). That hemophilia nevertheless does occur must be because the character arises comparatively often by mutation. As mentioned above one can, in point of fact, calculate the mutation frequency by investigating the number of persons with the character eradicated per generation. Provided that the frequency of the character is constant, the number of eliminated individuals must be met by mutations.

With regard to the actual occurrence of selection in human populations, we may mention that it has been strong in the case of defective individuals, particularly at an earlier date. They lived on the brink of starvation and had little chance of procreation. It can therefore be supposed that their frequency would be down at the least heterozygote limit. Moreover, I should like to remind readers that two big selection experiments have been made in human populations, one in a negative and one in a positive direction. The one is the celibacy of the Roman Catholic Church, which can

be said to have been an unintentional attempt to exterminate talent. Since talent is a rare character, the effect has probably not been great. If Catholic and Protestant countries are compared, *e.g.* England and France, no very perceptible difference emerges.

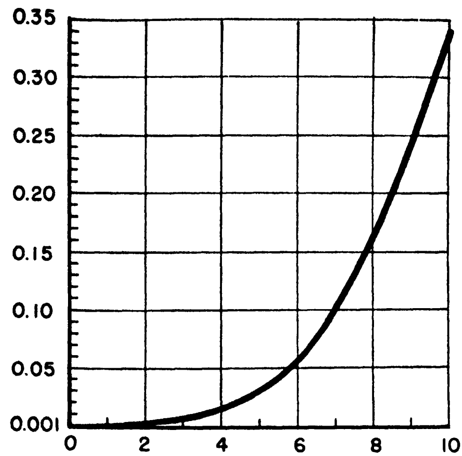


FIG. 7

Frequency of persons with a dominant character in successive generations at positive selection when their procreation is double ($k = -\frac{1}{2}$; *cf.* formulae 6 and 7) that of the corresponding recessive character.

The other great experiment is the polygamy found in Mohammedan countries in particular. Obviously, in practice it was primarily the wealthy who were able to indulge in the luxury of several wives. In as much as hereditary talent can be gauged by social success and riches, the propagation of these individuals has been favoured. It is a debatable point, what characters, if any, actually are favoured by this institution. Another debatable point, however, is whether it has had any very great effect.

IV. Assortative Mating

While selection and mutations alter the gene content in a population, the other deviations from panmixia only occasion a re-sorting of the genes, without changing their frequency. Assortative mating denotes the situation when persons with a character have a tendency to marry each other to a greater or a lesser extent than chance dictates.

The effect of total assortative mating was computed for the first time by Robbins (1917 and 1918). Koller and Geppert (1938) have submitted recursion formulae for partial positive assortative mating. Proceeding from other assumptions, the present author has given more general for-

mulae for partial positive assortative mating, which formulae can also be used to calculate the effect of negative assortative mating (Dahlberg, 1943).

If we call the number of generations n (the first generation is denoted 0) and the gene frequency r, the frequency of recessive homozygotes r_n^2 in total assortative mating in the nth generation is determined by the following formula:

$$r_n^2 = \frac{(n + 1)r^2}{1 + nr} \qquad (10)$$

and the frequency of heterozygotes in the nth generation, $2r_nd_n$, by:

$$2r_nd_n = 2rd - \frac{2nr^2d}{1 + nr} \qquad (11)$$

Such a process gradually leads to the disappearance of the heterozygotes and the population comes to consist only of the two different types of homozygotes (*cf.* figs. 8 and 9). The process begins quickly, and then goes more slowly towards this state of balance.

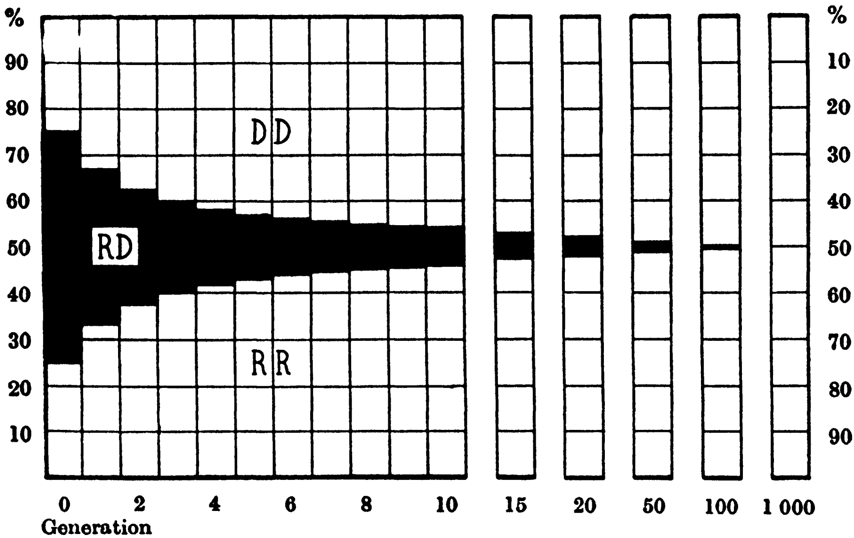


FIG. 8

Frequency of heterozygotes at total positive assortative mating in successive generations when a recessive gene has the frequency r = 0.5. *Cf.* formulae 10 and 11.

Now, total assortative mating can hardly exist in human populations; we need only concern ourselves with partial assortative mating. In positive partial assortative mating, the process is determined by the following recursion formula for the RR homozygotes:

$$r_{n+1}^2 = r^2 + \frac{2kd^2r_n^2}{1 - r_n^2} \qquad (12)$$

where k is half of the marriages which do not occur. The total number of marriages not contracted thus is = 2k of the marriages between persons with the character which would take place in panmixia. r_n^2 is the frequency of recessive homozygotes found after the process has been going on for n generations, r_{n+1}^2 is their frequency in the next generation, d^2 and r^2 is the frequency of the character at the beginning of the process. The above recursion formula can be used to calculate the frequency of persons with the character in consecutive generations.

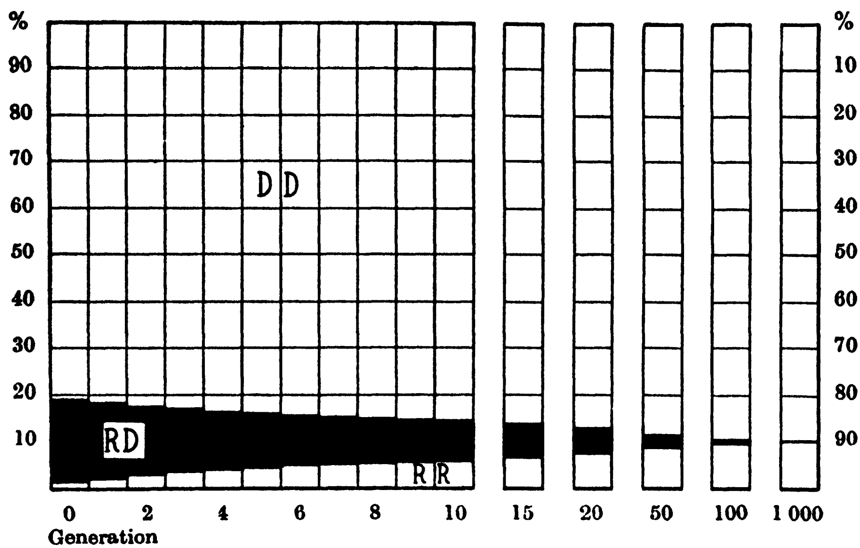


FIG. 9

Frequency of heterozygotes at total positive assortative mating in successive generations when a recessive gene has the frequency r = 0.1. *Cf.* formulae 10 and 11.

The following line of thought can be used to calculate the state of balance which has been reached (Dahlberg, 1943). We assume that one and the same process takes place when starting both from panmixia and from a population in which the heterozygotes have been eradicated by, for instance, total assortative mating. In the former case the heterozygotes will show a successive decrease, and in the latter a successive increase. The curves meet after a relatively small number of generations. In other words, it is possible in this way to show that a state of balance is soon reached (figs. 10 and 11).

Negative assortative mating implies that the persons with the character marry one another to a lesser extent than chance dictates. We can, for example, imagine (or hope) that obstinate persons intermarry less than is called for by chance. This character means, of course, that the

parties have less prospect of agreeing. That is to say, if obstinacy were a hereditary character, negative assortative mating would apply. The above formula 10 is used to calculate the effect, but k must naturally then have a negative sign.

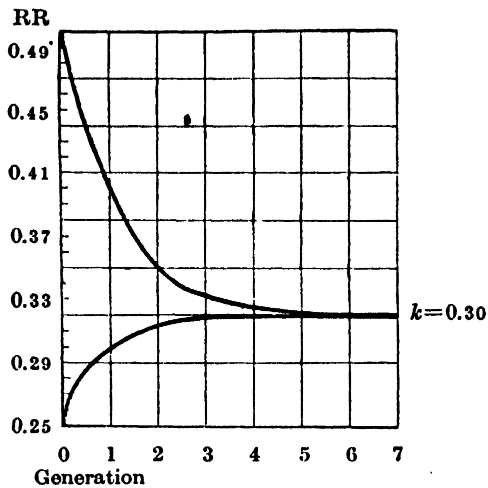


FIG. 10

Effect of positive partial assortative mating in successive generations with the intensity k = 0.30, when the frequency of the recessive gene is r = 0.5. In the calculations we have started from panmixia and also assumed that because of total assortative mating at the start there are no heterozygotes in the population. *Cf.* formula 12.

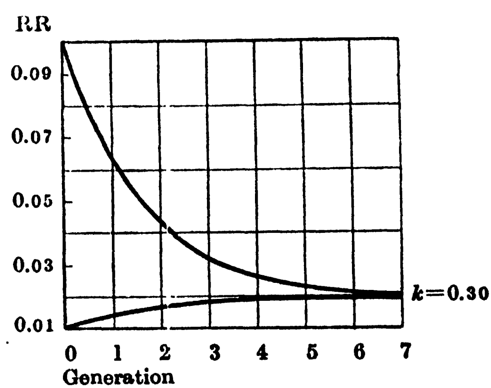


FIG. 11

Effect of positive partial assortative mating in successive generations with the intensity k = 0.30, when the frequency of the recessive gene is r = 0.1. In the calculations we have started from panmixia and also assumed that because of total assortative mating at the start there are no heterozygotes in the population. *Cf.* formula 12.

It may also be pointed out that the formula for negative total assortative mating is obtained by the introduction into formula 10 of:

$$k = -\frac{r_n^2}{2(1 - r_n^2)} \tag{13}$$

Here, too, the process approaches a state of balance very rapidly.

To calculate the effect of partial assortative mating, however, we can also assume that the marriages contracted by persons with the character form a constant proportion, h, of the marriages which would take place in every generation if marriages between persons with the character were contracted at random (Dahlberg, 1943). If r_n^2 is the frequency of recessive homozygotes in a certain generation, r_{n+1}^2 their frequency in the next generation, and r the gene frequency at the beginning of the process in panmixia, the general recursion formula will look like this:

$$r_{n+1}^2 = hr_n^2 + \frac{2r_n^2(r - r_n^2) \cdot (1 - h)}{1 - r_n^2} + \frac{(r - r_n^2)^2 \left[1 - \frac{r_n^2(1 - h)}{1 - r_n^2}\right]}{1 - r_n^2} \tag{14}$$

If $h = r^2$, there will be no change. The composition of the population is constant. If $h > r^2$, then there is positive assortative mating. If $h = 1$, there is total positive assortative mating. If $h < r^2$, there is negative assortative mating, and if $h = 0$, there is total negative assortative mating (fig. 12).

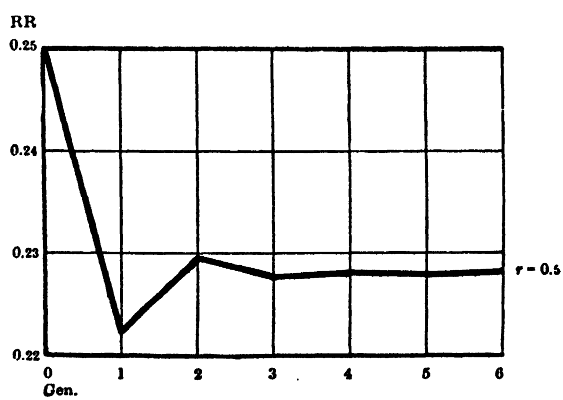


FIG. 12

Frequency of a recessive character at total negative assortative mating when the frequency of the recessive gene is 0.5. *Cf.* formula 14.

This formula, too, shows that a state of balance is soon reached both in positive and negative assortative mating. The effect of such processes is very moderate, even if there is assortative mating to a comparatively

high per cent. We do not at present know enough about assortative mating to be able to decide if the assumptions behind the last-mentioned formula are more in accordance with reality than those on which formula 12 is based.

Our empirical knowledge of processes of this kind in human populations is very limited. It has been found, for example, that tall persons are not inclined to marry short ones. As Pearson and Lee (1903) have shown there is a faint tendency towards positive assortative mating as regards stature. Characters such as musical talent may, in so far as they are due to heredity, be assumed to make for positive assortative mating, as also all hereditary characters whatsoever that can be thought to direct the interests in a given direction, since these interests form a bond between the respective parties which should promote their marriage. It can also be assumed that criminal individuals, for example, who may thus suffer from an inherited lack of judgment and balance, tend to marry one another. In so far as we are dealing here with rare characters, the possibility of assortative mating should have been on the increase up to the present, due to the development of communications, removal to the towns, etc. — in brief, by enlargement of the isolate. For that matter, assortative mating proceeds to a certain extent via isolates of special kinds. We shall be returning to this question.

V. Intermarriage

Intermarriage has the same effect as assortative mating. Extreme intermarriage, *i.e.* marriage between siblings, which is continued through several generations in crossing experiments, gradually eradicates the heterozygotes; this was first shown by Mendel and later mathematically worked out in more detail by Sewall Wright. A process of such intensity does not occur in human populations, however, though inbreeding of this kind was practised in the royal line of the Ptolemies in ancient Egypt and in the Inca of Peru. A certain frequency of blood marriage occurs in panmixia, so that in calculating the effect of intermarriage, regard must be paid to the size of the population and to the degree of intermarriage which is to be expected from chance. In small populations and large families we should expect a certain frequency of marriages between siblings, which is not the case in human populations. It can, however, be shown that the deviation this causes is but slight.

The fact that evolution has taken place implies that all marriages are blood marriages, even though very distant. As already pointed out, panmixia presupposes in all cases a certain degree of intermarriage. Intermarriage can be said to take place if more blood marriages are found than

are to be expected in panmixia. (If the frequency of the blood marriages is lower than was to be expected, we could call this "outbreeding.")

In very large populations, the frequency of blood marriages to be expected from random mating is extremely small. In practice, however, marriage between cousins in such populations must be expected to a greater extent than chance would suggest. If such marriages are assumed to take place to 100%, *i.e.* if the contracting parties are always cousins, the deviation from panmixia after one generation can be calculated according to the following formula:

$$\frac{1}{16}(r - r^2) = \frac{2rd}{32} \qquad (15)$$

which means that the heterozygotes diminish by 1/16, and that this diminution is compensated by an increase of the two kinds of homozygotes (Dahlberg, 1929). The extent of this increase, then, depends on the gene frequency. It is greatest if $r = \frac{1}{2}$. In this case the recessive homozygotes in panmixia will constitute 25%. In one hundred per cent cousin marriages, their frequency will be 26.56%. In other words, the increase is 1.56%. The increase obtained in different gene frequencies appears from fig. 13.

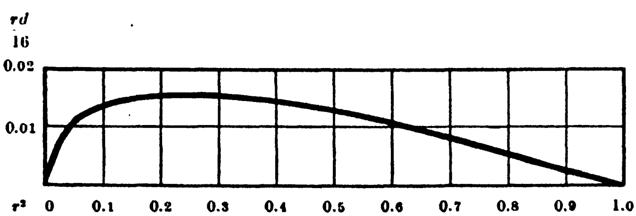


FIG. 13

Increase of the frequency of a monohybrid recessive character in a population where only marriages between cousins are contracted compared with panmixia at different frequencies of a recessive gene. To make the curve visible the scale is ten times larger in vertical than in horizontal direction. *Cf.* formula 15.

As is seen, it is very moderate. Now, there is no population with one hundred per cent marriages between cousins. The normal frequency for such marriages in the larger civilised countries is probably less than ½%. We can therefore say that the increase in a character's frequency to be expected in practice in human populations has no important bearing on the degree and frequency of intermarriage. (However, intermarriage does not affect only one gene in a population; it affects them all and from this viewpoint is by no means negligible. See below.)

We have now examined the effect of intermarriage on a monohybrid character from a populational viewpoint. If we look at this effect from

the viewpoint of the persons with the character, we get another result. If a gene is very rare, that is to say, has sprung from a single mutation in a population, it can come together in homozygote form only by a blood marriage. If the character is a usual one, intermarriage proves to be of no consequence. If, on the other hand, it is rare, intermarriage means everything, but the character then has no importance for the population.

For an idea of the importance of intermarriage from the viewpoint of the persons with the character, it will be simplest to calculate the proportion of persons with the character whose parents were cousins. An approximate formula for this has been given by Lenz (1919). The correct formula has been given by Dahlberg (1929), and runs as follows, where k implies the proportion of persons with the character whose parents are cousins, r the gene frequency, c_1 the actual frequency of marriages between cousins, and c_2 the frequency of such marriages to be expected in panmixia:

$$k = \frac{c_1}{c_1 - c_2 + \frac{16r(1 - c_1 + c_2)}{1 + 15r}} \qquad (16)$$

The formula shows that the rarer the gene in the population, the greater the number of persons with the character springing from marriages between cousins (fig. 14). Everything else being equal, this proportion will naturally be higher in a population where such marriages are common, than in one where they are rare.

One consequence of this is that if characters are rare, they are often found together among the children of parents who are cousins. This concurrence may be wrongly taken to express linkage.

If k_1 is the proportion of persons with the character springing from married cousins in respect to the one character, k_2 the proportion in respect to the other, and c the frequency of marriages between cousins in the population, the relation between the real and the expected frequency of the coincidence of two characters in one and the same individual in a population (y) will be given by the following formula (Dahlberg, 1943):

$$y = \frac{(1 - k_1)\,(1 - k_2)}{1 - c} + \frac{k_1 k_2}{c} \qquad (17)$$

The formula shows that this mechanism has a noticeable effect only on very rare characters. Other circumstances being equal, the effect is naturally greater when the marriages between cousins are common than when they are rare. This effect of blood marriages also leads one to expect that siblings of persons exhibiting a rare hereditary defect will show this and other rare defects to a somewhat higher frequency than is the case in an average population.

As intermarriage somewhat increases the frequency of homozygotes but decreases the frequency of heterozygotes, this means that negative selection has a greater effect in a population showing a high intermarriage frequency than in one with a low one. It also means that more recessive mutations become manifest in a population with a high intermarriage

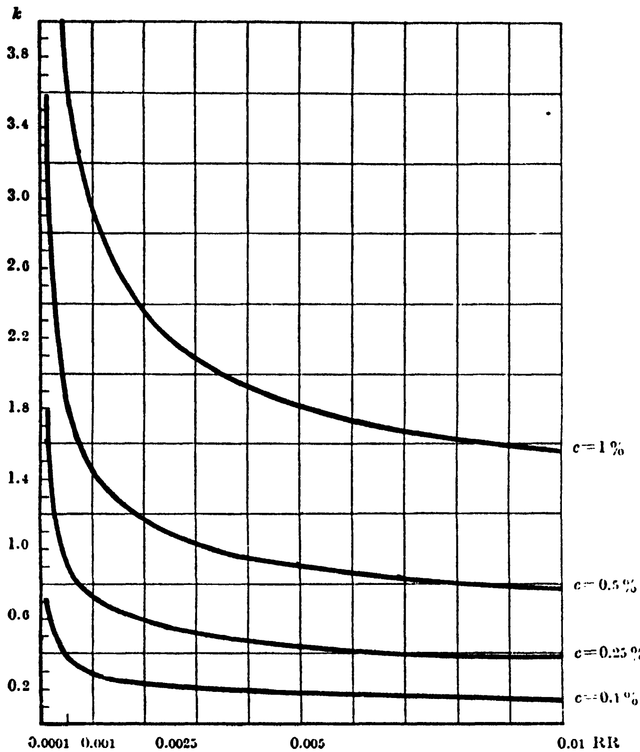


FIG. 14

Percentage of persons with a recessive character deriving from marriages between cousins (k) in populations where the frequency of marriages between cousins (c) and the frequency of a recessive character is varying. *Cf.* formula 16.

frequency than in one with low. In other words, if the intermarriage frequency of a population is lowered, the state of balance between negative selection and mutations will be upset. The least heterozygote limit is raised when intermarriage falls, which means that the gene content will rise very slowly, due to mutations, before equilibrium is again reached. In principle, of course, intermarriage works in the same way as assortative mating.

VI. Isolates

A population can be said to consist of partial populations of isolates, in which there is panmixia. The situation here is naturally very different in different populations. Isolates can be of two kinds, geographic and social. Geographic isolates were specially common in European populations of earlier ages, where the population of a country consisted of settlements and villages separated by forests, etc. It may be remembered that the people usually lived by water-courses, and that lakes and rivers formed links of communication, whereas forests and mountains formed dividing barriers.

The social isolates may be conditioned by economic factors, but religious elements and traditions of other kinds may cause a certain group of individuals to intermarry and keep apart from the rest of the nation.

1. *Size of the Isolates*

If marriages between cousins are contracted at random, their frequency will obviously come to depend partly on the average size of the isolates and partly on the average size of the family. Conversely, then, the size of the isolates can be reckoned if we know the frequency of the cousin marriages and the average size of the families.

If the size of the isolate is called n, the average number of children becoming adult and marrying b, and the frequency of cousin marriages c, we have the following formula given by Dahlberg (1938):

$$c = \frac{2b(b-1)}{n-1} \quad \text{and} \quad n - 1 = \frac{2b(b-1)}{c} \tag{18}$$

If we assume that the numbers of the population remain fairly constant, b must be equal to 2. The average size of the isolates in different frequencies of cousin marriages is seen in Table 1. Needless to say, the values there

TABLE 1

Size of the Isolates (= n), when Marriages between Cousins are Contracted at Random in Different Frequencies (= c). *Cf.* Formula 18

c	*n*
0.0001	40 000
0.001	4 000
0.0025	1 600
0.005	800
0.01	400
0.05	80
0.1	40

are highly approximate. An isolate very seldom has clear-cut boundaries. We can often say that it merges gradually into the population, and that every person has his or her own isolate. The isolate calculated by the above formula is in point of fact often fictitious: the calculated isolate is a sharply demarcated one, which would give the same frequency of marriages between cousins as the real and less clear-cut isolate.

The above formula presupposes that marriage between cousins is contracted at random and is solely dependent on the size of the isolate in relation to the size of the family. In populations where the parents arrange the marriage of the children (as was once specially common in France, for example), we can expect marriage between cousins to show a far greater frequency, and this frequency to remain more constant than would otherwise have been the case. In many populations, however, the frequency of such marriages has decreased up to the present time, due to the breaking of isolates and to smaller families.

2. *Isolates and Heterozygote Frequency*

Obviously, the heterozygote frequency obtained if panmixia is assumed in a population is far greater than that found if the population is divided up into isolates. In other words, we get a wrong gene frequency if we calculate it with the help of the known frequency of recessive characters and assume panmixia. The correct frequency, r_t, is obtained from the following formula, submitted by Wahlund (1928):

$$r_t = \sqrt{r^2 + \sigma_i^2} \tag{19}$$

where

$$\sigma_i^2 = i_1(r - r_1)^2 + i_2(r - r_2)^2 + i_3(r - r_3)^2 + \cdots i_m(r - r_m)^2$$

In this formula $i_1 i_2 i_3 \ldots i_m$ denotes the size of the different isolates, and their gene frequency is shown by $r_1 r_2 r_3 \ldots r_m$, while r gives the frequency of the R-gene in the total population obtained by the formula for panmixia.

If an ordinary character is in question, it is hardly likely that the gene content in the different isolates would cause greater differences from those to be found in panmixia throughout the entire population. In the question of rare characters, on the other hand, it is likely that the relevant gene only occurs in a few isolates and is completely absent from the others. Such rare genes become manifest — *i.e.* occur in homozygote form — primarily in small isolates, *e.g.* remote villages, in which the frequency of intermarriage is relatively high. The appearance of these rare characters is often laid at the door of intermarriage and the population is called degenerate. It is, however, more correct to say that lack of communications is the real cause. In any case too high heterozygote frequency and too

high gene frequency are obtained if panmixia is assumed for a rare character which only occurs in certain isolates.

3. *Breaking of Isolates*

If we envisage a single isolate in a population where there is a certain frequency of a gene causing a defect, lacking in other isolates, it is plain that marriage beyond the isolate boundaries is harmless, since it is not likely that the gene is present in the neighbouring isolates. For this reason, a breaking of the isolate will lead to a lowering of the homozygotes — in other words, a lowering of the frequency of rare hereditary defects, and an increase of the heterozygote frequency. If the gene in question has the frequency r, and if the isolate is enlarged by a part, a, of itself, the frequency of the persons with the character will be:

$$RR = \frac{r^2}{1 + a} \qquad (20)$$

In other words, the frequency of the character diminishes in proportion to the expansion of the isolate, so that if the isolate is doubled, the number of persons with the character decreases to half their original number (Dahlberg, 1938).

Now we know that the frequency of marriages between cousins was formerly much higher than now. In Bavaria, for example, it was 0.20% in 1926–33, while it was 0.87% 50 years earlier. This decrease is due partly to the fact that fertility and size of families has fallen somewhat, but above all to the fact that the development of communications, industrialisation, and migration to the towns has caused a breaking of the isolates. This process must materially have lowered the frequency of hereditary defects in civilized countries up to our own time. As far as the frequency of hereditary defects is concerned, the attempt to keep the population on the land is naturally of doubtful value.

If, then, the frequency of marriages between cousins prompts us to assume that the breaking of isolates has caused, say, a doubling of the size, this should have led to a halving of the frequency of the rare defects, such as hereditary blindness, mental deficiency of different kinds, etc.

If there is only a certain interchange beyond the boundaries of the isolate, the difference between isolates will take time to level out. The formula for the speed at which this levelling-out takes place has been given by Wahlund (1928), who has also given the formula for the process in polyhybridism. If we assume that the isolate limits are abolished, so that free crossing took place, we would at once — *i.e.* in the next generation — get that composition of the population for monohybrid characters which is to be expected in panmixia. When polyhybrid characters are concerned,

however, the genes will accompany one another to a certain extent. The connection will only gradually be dissolved, giving the composition to be expected in panmixia. This process takes many generations.

To take a practical example, it may be mentioned that the increase in stature which has been noticed in several countries, and which in Sweden amounts to 9 cm in 100 years, can probably be mainly attributed to isolate breaking (Broman, Dahlberg and Lichtenstein, 1942).

4. *Isolates and Race*

It is customary to define a race as a group of individuals who show deviating hereditary characters from other groups. Now this definition needs clarifying in several respects. For the moment we can only dwell on what is meant by a group in this connection. In a population, tall and short individuals can be differentiated according to some more or less arbitrary norm. But we do not consider the tall ones to constitute a separate race, provided they cross freely with the short ones. It is only if the tall individuals keep to themselves, *i.e.* form an isolate or a group of isolates, that we may be inclined to discuss whether they should not be regarded as a separate race. The isolate conception therefore in some ways lays the foundations for the conception of race (Dahlberg, 1942).

If the members of an isolate or a group of isolates are to be regarded as a separate race, they must naturally show deviations from the members of other isolates. Strictly speaking, one cannot talk of racial characters, but only of racial differences. A character which is not a racial character today may become so tomorrow if a race is discovered without it.

It is true that two races can be so completely different that it is possible to say with certainty whether an individual belongs to one or the other (*e.g.* negroes and Mongolians). When speaking of races which partly have a common range of variation, however, it is necessary to find a gauge for the degree of the difference. A suitable method is first to study the variability of the characters to be used in the two groups to be compared. The individuals of both groups are then imagined mixed together, and the knowledge one has of the separate groups is then used in trying to sort them out again correctly; one can, of course, relegate to the one group those individuals falling outside the other's range of variation. The sorting out is done, first according to single characters, and then according to combinations of characters (Dahlberg, 1941). The percentage of individuals correctly sorted out can be used as a measure of the size of the race difference. Another way is to use the information obtained for making the best possible dividing up of the mixed groups. It must then be remembered that in random sorting 50% can be expected to be correctly sorted out. The amount by which the empirical percentage exceeds 50 must therefore

be a gauge of the racial difference. The racial difference coefficient drawn up by Dahlberg therefore has the following form:

$$k = \frac{50 - p}{100} \tag{21}$$

where p is the percentage which can be differentiated with certainty.

We may point out that when separating individuals of different races, the chances are better if several characters are used. Not much is gained, however, by increasing the number of investigated characters, if there is correlation between them. The smallest number, N, of characters required to sort out all individuals is obtained from the following formula:

$$N = \frac{9(1 - r)}{a^2 - 9r} \tag{22}$$

In this formula, r is the average correlation and a the average difference between the means of the races for the different characters (fig. 15).

If there are racial differences within a people, this must cause a

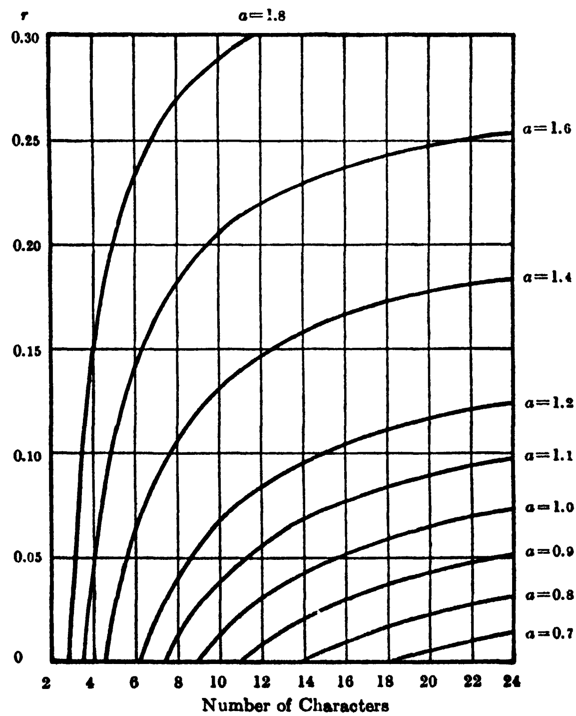


FIG. 15

The smallest number of characters required to differentiate each individual from two amalgamated groups at different degrees of average correlation (r) and average difference between the means (a). *Cf.* formula 22.

correlation between characters. If we find that there is no correlation between physical characters, it therefore means that there are no grounds for assuming different racial groups within a population, even if it originally sprang from different races. It is homogeneous. Naturally, we must here build on characters that are not occasioned by the same genes. For example, there is a correlation between the colour of the hair of the head and that of the body, which is contingent on the same genes; needless to say, such characters cannot be used in this connection. The correlation between genes diminishes one-half per generation, as has been shown by Wahlund (1932). This implies that racial differences are very quickly smoothed out by free crossing, *i.e.* by isolate breaking.

5. *Isolates, Mutations and Random Selection*

Recessive mutations will come to the fore more quickly in a population built up of small isolates than in one composed of large ones. When a recessive gene arises by mutation, it will only after some time occur in a "double dose" by means of intermarriage — soonest by a marriage of cousins. In other words, when the character appears, the gene has acquired a certain frequency in heterozygote form. That is to say, the frequency of the gene can never be pushed below this frequency (least heterozygote frequency) by negative selection — always assuming that the isolate in question is not very small or the frequency of the gene extremely low, since in that case the gene may disappear at random.

There is always a certain likelihood of a mutation disappearing, after once arising. The person or persons with the new gene may not procreate, and then of course the gene disappears from the isolate. A risk of this kind may also extend to future generations. On the other hand, a gene may be lucky and, by chance, have a very large circulation. It is easier in small isolates than in large for the gene to increase at random until it is found in all the individuals there. These possibilities were first pointed out by Hagedoorn (1921). As previously mentioned, the two main investigators to treat this problem from a mathematical aspect are Sewall Wright and R. A. Fisher. It is plain that the mechanism indicated here will cause differences between the isolates making up a population.

The random selection mechanism also means that, when polyhybrid characters are concerned, the frequency of persons with the character diminishes. If a character is contingent on several pairs of genes, it will be easier for some of the pairs, and therewith the prospects of the character, to disappear if the isolate is small. Different pairs of genes can in this way be expected to disappear at random in different isolates. The genes occasioning the character can then only come together after a breaking of the isolate. The same is true of polymeric characters. Some of the

polymeric genes will disappear, others will increase, until they embrace the entire isolate. This process will diminish the difference between the isolates. When the isolate is broken, the polymerically conditioned character will have greater variability, while the mean will remain unchanged.

6. *Isolates, Intermarriage and Selection*

It has already been stated that if a recessive character is rare, the individuals showing this character will to a relatively large extent be the children of cousins. If the character is very rare and the frequency of cousin marriages high, this situation will be particularly marked. When the population is divided up into isolates, however, it will mean a restriction of the possible differences. Very rare genes cannot occur in small isolates, since there must be at least two heterozygotes possessing the gene for the character to appear. It is therefore only in large isolates that a gene can be very rare. In large isolates, *e.g.* a capital, however, we cannot expect a high frequency of marriages between cousins. For this reason, the two factors, the gene frequency and the frequency of marriages entailing parents who are cousins will balance one another, so that the frequency of cousin marriages to be expected among the parents will be between 15% and 30% (Dahlberg, 1938). This theoretical result has been verified empirically. The frequency of marriages between cousins among parents of individuals with rare defects keeps at the calculated per cent. In juvenile amaurotic idiocy, for example, it is 15%, in retinitis pigmentosa 17%, in Friedreich's ataxia 9%, in albinism 17%, etc.

This means that by forbidding marriage between cousins, the frequency of persons with the character can at best be lowered by 15%, but hardly by much more. The gain thus achieved is reached in a generation: there will be no change after that. On the other hand the process affects all rare characters, in the population, above all those with monohybrid recessive heredity. It does not act so strongly on the polyhybrid characters.

7. *The Rise of Isolates*

While the gene content of a population is changed by mutations and selection, assortative mating and intermarriage — as already mentioned — cause only a decrease of the heterozygotism, without changing the gene content. The appearance of isolates has the same effect in regard to inherited characters which appear only in a few of the isolates. Conversely, isolate-breaking in this situation leads to an increase of the heterozygotism up to the level characterizing panmixia.

Isolates of different make-up can arise in several ways. The cause may be selective emigration. When a new country is populated, *e.g.* America after about 1500, the immigration on which the population is

contingent is doubtless often of a selective nature. Moreover, the new country gives rise to isolates which are bound frequently to be of different natures. If the new isolates are small, they may happen to acquire different characters at random. We know nothing of the extent to which populational differences between European countries are occasioned by prehistoric immigration of a similar kind. In regard to isolationism in the U.S.A., we know very little about where it is going to lead us.

Another possible cause of differences between isolates is the process by which social classes arise in a population. It is stated that the upper class is entitled to its favourable position by birth — that is to say, the upper class has arisen by a selective process, and contains a greater number of particularly able persons than the lower classes. However, we know very little of the origins of the upper class, but it is interesting to make a theoretical analysis of an assumed selection process of this kind.

The simplest way is to assume that the population consists of only two isolates, isolate A (the upper class), which is comparatively small, and isolate B (the lower class) which is comparatively large. We will further assume that, initially, the isolates have the same frequency of a recessive gene, *r*, and that a certain proportion, k, of those possessing the character are transferred from B to A in each generation. The frequency which the character will have in isolate A is determined by the following recursion formula (Dahlberg, 1943). If the frequency in a given generation is x_n^2, the frequency in the following generation will be:

$$x_{n+1}^2 = \left[\frac{(A_n x_n + B_n k r_n^2)}{A_n + B_n k r_n^2}\right]^2 \qquad (23)$$

where r_n^2 is the frequency of the character in isolate B. This frequency is determined by the previously given formula for partial selection (formula 4). That is to say, there is partial selection here, which under all circumstances should have very little effect, as the isolate is relatively large. The significance this selection process will have for isolate A depends on the intensity of the process, *i.e.* of the constants included in the above formula. Now, let us assume isolate A (the upper class) comprises, say, 5% of the population, that the character subjected to selective transference from one isolate to the other has the frequency of 1% at the time the isolate arose, and that the selection covers 10% (k = 0.1) of the persons with the character (*i.e.* 10% are transferred to isolate A in each generation), then in 10 generations the frequency will rise to 2.4% in A. If the selection only covers 1% (k = 0.01) of the persons with the character, the increase will be infinitesimally small, namely 1.1% per 10 generations. The corresponding effect in isolate B (lower class) with a selection of 1% (k = 0.01) will be a fall in the frequency of the character from 1% to 0.99%. If the selec-

tion is 10%(k=0.1), the frequency of persons with the character will fall in the same time to 0.94%. These figures show that the selection has little effect on the lower class, but may sometimes but not always noticeably affect the make-up of the upper class (fig. 16).

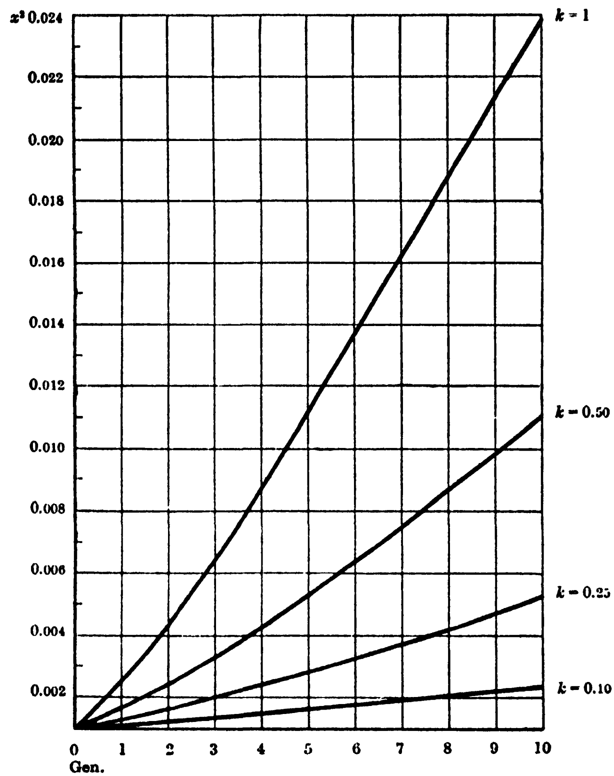


FIG. 16

Composition of the upper class in successive generations when it embraces 5% of the population and the persons with the character at the start have the frequency of 1°/$_{\infty}$ and when the transference to the upper class has the frequency 10% (k = 0.1) to 100% (k = 1) according to formula 23.

As previously mentioned, however, we have no empirical constants for the class circulation, so that calculations have as yet a solely theoretical interest. They might conceivably be thought of importance in that they show the impossibility of making any pronouncement about class differences that is based on real knowledge of what happened and is happening.

Even if the upper class originated from selection, there is naturally no reason why we should be satisfied with the effect achieved. If an upper class of particularly high quality is wanted, the simplest thing should be

to give everyone the same chance of working up to a leading position. Genetically speaking, this would mean a gain, since the selection would then have a wider scope than if it were made only from a smaller part of the population.

8. *Assortative Mating, Intermarriage and Isolates*

As has already been mentioned, assortative mating, intermarriage and isolates change the frequency of a gene, in that the genes are given another distribution than in panmixia. The deviation is due to the fact that the marriages which are contracted are determined in a certain way. It is therefore clear that it may sometimes be a matter of taste whether one talks of one process rather than another. As regards negroes in the U.S.A., for example, we can talk of the effect of isolates, but we can equally well say that there is assortative mating, caused by a special psychological attitude in the population.

When dealing with certain defectives, *e.g.* deaf-mutes, blind, etc., we can similarly assume the effect of isolates, but we can also claim a certain degree of assortative mating. The defectives are brought together by societies, so that they have contact and marry to a particularly large extent. Their physical disabilities put difficulties in the way of marriage with others. In other words, the defectives form an isolate which has the peculiarity of being newly formed for each generation. Formulae have been drawn up for defective isolates of this kind, but they build on very uncertain empirical foundations (see Dahlberg, 1943). As the defectives are undoubtedly less fertile than the average population, we can say that there is selection in combination with isolates which are formed by assortative mating.

It is naturally also a matter of taste whether we attribute the heterozygote increase taking place in present-day populations to the breaking of isolates or to less intermarriage. The view taken in individual cases must depend on such things as the empirical points of departure we have at our disposal.

VII. Summary

Human genetics have arisen from animal and plant genetics. In Germany this branch of research came to be influenced by ideas about race, as can be seen, for example, in the German term Rassenbiologie. In that country it gradually came to enter into, and be influenced by, the Nazi ideology.

In England, human genetics came to be linked with Galton's eugenics (the doctrine of the well-born), and was therefore extensively directed

towards investigations of families considered to be of a particularly high quality.

Thus, with human genetics focussed on population problems connected with politics as it was in England and Germany, no very important research was done. The main result from this point of view was the subjective coloured propaganda literature without any scientific value worth the name.

However, both Germany and England produced many valuable detail investigations bearing on the heredity of normal and pathological characters, though there was perhaps too great a tendency to imitate the crossing experiments that had been made in animal and plant genetics. Now, human subjects cannot be crossed in order to see what the result will be. Instead, family investigations were made, from which were evolved pedigrees which, formally, bear a certain similarity to crossing experiments. But this is beginning at the wrong end — that is to say, the start is made not from the so-called first ancestors but from individuals now living, who are related and who show an accumulation of a certain character. From these, one proceeded backwards in time. The result a certain crossing would have on the human subject was not studied; it was to a certain extent a foregone conclusion, thanks to the selected initial material. In spite of this, important results have beyond any doubt been obtained, and a fund of knowledge collected as to how different characters, in particular more or less rare diseases, are inherited.

Meanwhile, a few investigations into the mechanism behind changes of population have been made. They are theoretical and mathematical in character, and are to be found in the most varied periodicals. I have tried to review these investigations in the present paper, since it seems to me that such studies will have particular importance for human genetics in the future. Before attacking the problems practically, however, it is necessary to have a firm theoretical point of departure for the avenues of approach that are laid down. Mathematical investigations are also of importance, as they counteract the more or less incongruous constructions that are universally circulated in this field.

But necessary though it may be to develop the theoretical-cum-mathematical side of the problems, the primary need is for empirical investigations of the processes taking place in human populations. We require both knowledge of the frequency of intermarriage, assortative mating, the formation of isolates, etc., and also investigations of the actual frequency of individual characters in populations. We have, however, still very little possibility of comparing the make-up of a population at different junctures, or of comparing different populations at the same juncture. A great deal must be done to achieve an empirical foundation

for the assessment of populations from the viewpoint of heredity. But this must be regarded as a very important task for human genetics to carry out.

VIII. REFERENCES

Andreassen, M., Haemofili i Danmark. Ejnar Munksgaard, København (1943).

Broman, B., Dahlberg, G., and Lichtenstein, A., *Acta paediatr., Stockh.* **30,** 1-66 (1942).

Dahlberg, G., *Genetics* **14,** 421–454 (1929).

Proc. roy. Soc. Edinb. **58,** 213–232 (1938).

Die Vererbung der allergischen Disposition. P. Kallós; Fortschritte der Allergielehre. S. Karger, Basel and New York. (1939).

The conception of race and a new method of delimiting races, demonstrated on a material of Swedes and Lapps. G. Dahlberg and S. Wahlund: The race biology of the Swedish Lapps. Part II. Almqvist and Wiksell, Uppsala. (1941).

Hum. Biol. **14,** 372–385 (1942).

Acta med. scand. Suppl. **148** (1943).

Zool. Bidr. Uppsala **25,** 21–32 (1947).

Mathematical Methods for Population Genetics. S. Karger, New York (1947).

Dahlb erg, G., and Hultkrantz, J. V., *Arch. Rass.-u. Ges. Biol.* **19,** 129–165 (1927).

Hagedoorn, A. L., and Hagedoorn-Vorstheuvel La Brand, A. C., The relative value of the processes causing evolution. Martinus Nijhoff, The Hague (1921).

Haldane, J. B. S., *Trans. Camb. phil. Soc.* **23,** 19-41 (1924).

J. Genet. **31,** 317-326 (1935).

Hardy, G. H., *Science* **28,** 49–50 (1908).

Koller, S., and Geppert, H., Erbmathematik. Quelle und Meyer, Leipzig (1938).

Lenz, F., *Münch. med. Wschr.* **66,** 2 (1919).

Mørch, E. T., Chondrodystrophic dwarfs in Denmark. Ejnar Munksgaard, København (1941).

Pearson, K., *Philos. Trans.* **203,** 53-86 (1903).

Pearson, K., and Lee, Alice, *Biometrika* **2,** 357–462 (1903).

Robbins, R. B., *Genetics* **2,** 000 (1917).

Genetics **3,** 000 (1918).

Sjögren, T., Die juvenile amaurotische Idiotie. Hereditas, Lund **14,** 197–425 (1931).

Sköld, E., *Acta med. scand.* Suppl. (1944).

Wahlund, S., *Hereditas, Lund* **11,** 65–106 (1928).

Demographic studies in the nomadic and the settled population of northern Lapland, Uppsala (1932).

Genetics of Cancer

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CONTENTS

I. INTRODUCTION

A cancer is a physiologic character involving growth processes. Like other physiologic characters, it is to be viewed as the result of a chain of processes directed by both environmental and genetic factors. None of the factors of either group can be considered as the sole cause of the malignancy, but each factor must be considered as influencing the probability that cancer will occur.

The past forty years of inbreeding and hybridization studies have led us to consider that genetic differences can be expected to be revealed in the study of the physiologic complex that precedes the formation of almost any type of cancer. Advances in other fields of physiologic genetics also compel such an attitude, particularly those advances being made in the new field recently reviewed by Beadle (1945) in which the geneticists and the chemists are joining forces in demonstrating the relationships between the gene and the enzyme. When by this work we are forced to the realization that metabolism in general and protein metabolism in particular are under genic control, we can not conceive of a physiology preceding

malignancy that does not include genic action. And when we consider that genes do mutate, we must expect that genic differences will occur in the physiology leading to the formation of the cancer.

Because of these developments, our concept of the problem has evolved into one not so much concerned with proving whether or not cancer is inherited but in ascertaining how various genic patterns affect the probability that it will appear and how these patterns are inherited. From the extensive inbreeding work we now have a wealth of material in which genic differences have been and are being demonstrated. The next step is to determine where these differences are and how they become manifest. What are the gene action paths? Such an approach together with investigations in other fields of physiological genetics, will lead us ultimately to a knowledge of the specific gene action and how this action fits into the physiology leading to the cancer. No other approach has proved more fruitful in contributing to our understanding of the cause of cancer.

In the following sections the contributions to our present knowledge of the genetics of cancer are reviewed. No attempt is made to make the review complete, and emphasis is placed on the more recent work. Both the so-called spontaneous and induced tumors are considered since in many cases the same genic differences appear to influence both. The genetics of tumor transplantation is not covered, for it is a separate subject worthy of separate consideration.

II. Inbred Lines

Practically all experimental work on the inheritance of cancer today is dependent upon inbred lines of experimental animals, and thus any review should include a discussion of the development of this foundation material.

As early as 1909, Little foresaw that to analyze any character as complicated as cancer appeared to be, it would be necessary to have genetically controlled material. The value of this attitude was soon to be verified by the validity of the interpretations which he was in position to give as compared with those arrived at by Slye, using heterogeneous material. It was in 1909 that Little initiated the inbreeding program by starting the well-known high-mammary-tumor strain dba of mice. Around 1920 a number of other strains were started by Little and by Strong, and later these strains together with the older strain dba formed the foundation on which the Roscoe B. Jackson Memorial Laboratory took the lead in this field of endeavor (Heston, 1945; Snell, 1941; Strong, 1942). The program expanded not only at the Jackson Laboratory but also elsewhere, until today there are around seventy or more isogenic strains of mice approximately half of which have been used in cancer research. Work is con-

tinuing on the development of new strains, one of the most interesting of which is the high-gastric-cancer strain of Strong, which is discussed in detail later.

The development of isogenic strains for cancer studies has not been limited to the mouse. Dunning and Curtis (personal communication) have contributed in a major way to the development of such strains of rats. They have approximately a dozen inbred strains which they have used in their specific problems in cancer research. The Albany group has developed an inbred strain of rats with a high incidence of adenofibroma of the mammary gland (Bryan, Klinck and Wolfe, 1938). Two of Wright's original inbred strains of guinea pigs (families 2 and 13) are now being maintained at the National Cancer Institute, where they are regarded as of particular value since tumors induced in them can be carried by transplantation. In addition programs are being undertaken for the development of inbred strains of still other species including rabbits, hamsters, chickens, and dogs. Because of the size of some of these animals, however, it requires greater courage and financial support to undertake the task of developing isogenic strains of these species. Nevertheless it is a task which must be undertaken and carried through, if medical research, cancer research in particular, is to progress as we hope it will.

The tumor-incidence data for these inbred strains have been one of the major sources of information on the inheritance of cancer notwithstanding the fact that many of these strains were developed in different laboratories, and therefore the data were gathered under a variety of environmental conditions. A number of points can be exemplified by the data given in Table 1.

In the first place, the tumor incidence data demonstrate that genetic factors are involved. It is recognized that in the process of inbreeding, nongenetic factors influencing the appearance of a tumor may be built into a strain. The milk factor is an example of this. But it is safe to assume that the differences in the tumor incidences of these strains are probably for the most part genetic. As pointed out later, even the establishment of the milk factor in a strain appears to have a genetic basis.

The second major point established by the production of these inbred strains is that not all cancer is controlled by the same set of genes, but that generally different types are inherited as separate characters. If one considers the data presented in Table 1, he notes that the strains cannot be divided into two groups, cancerous and noncancerous, but that each strain is characterized by a specific type or types of cancer. Strain C3H has a high incidence of mammary-gland tumors but a low incidence of pulmonary tumors. It does have a relatively high incidence of hepatomas. Strain A has a high incidence of mammary-gland tumors and also a high incidence

TABLE 1

Tumor Incidence in Some of the Inbred Strains of Mice Commonly Used

Strain	*Mammary tumor incidence in breeding females (per cent)*	*Lung-tumor incidence (per cent)*	*Leukemia incidence (per cent)*	*Remarks*
C3H (Andervont, 1941; Bittner, 1935, 1943)	75 to 100	5 to 10		Hepatomas: females 10 per cent; males 27 per cent
A (Bittner and Little, 1937; Bittner, 1938, 1939; Heston, 1942; Murray and Hoffman, 1941)	70 to 85	80 to 90	Low	
dba (Murray and Hoffman, 1941; Murray, 1934; Korteweg, 1936; Mider and Morton, 1939)	55 to 75	Low	30 to 40 (subline 212)	
C57 black (Bittner, 1938; Little *et al*, 1939; Bittner and Little, 1937)	< 1	< 1		
C57 leaden[1] (Heston, 1942, 1945)	< 1	< 1		
B alb C[2] (Andervont, 1938)	< 5	20 to 30		
I (Andervont, 1943; Andervont and Stewart, 1937; Shimkin, 1940)	< 1	10 to 20		Develop adenomatous lesions of the stomach
Swiss (Lynch, 1940; Snell, 1941)	19	40 to 50		
C58 (MacDowell and Richter, 1935)			90	
Rf (Cole and Furth, 1941; Snell, 1941)	Low	32	2	
Ak (ibid)	do	Low	60 to 80	

[1] Also known as strain L and strain M.
[2] Also known as strain C.

of pulmonary tumors. It does not have a high incidence of any other type. Strain C57 black has a low incidence of mammary-gland tumors and also of pulmonary tumors. About 20 per cent of the animals of the strain do develop tumors, however, most of which are of the leukemia group. MacDowell's strain C58 and Furth's strain Ak are characterized by high incidences of leukemia. Strong (1945) is developing a strain with a high incidence of gastric carcinoma. Woolley (Woolley and Little, 1945a and b) has a strain characterized by adrenal tumors following early castration.

These data indicate that specific genes are involved in the production of specific types of tumors and that there is not a common genotype favorable to tumors in general. This point is emphasized since it is not wholly in accord with some of the studies of the less controlled human material which indicate the possibility of the inheritance of a more general tendency to tumor formation (Jacobsen, 1946). The apparent situation in the experimental animals is more nearly what one might expect on the consideration of the physiology preceding the development of the tumor. From our present experimental evidence we must picture these genic differences as being manifest through the physiology leading to the tumor formation, and this physiology is not the same for all types of tumors. Sex hormones play a very prominent role in mammary tumors, but there is no evidence that such hormones play a similar role in pulmonary tumors. One would not expect the physiology that precedes gastric carcinoma to be the same as that preceding adrenal tumors. Thus, we cannot expect common genic differences for all types of tumors, but with certain reservations, we must expect the different types to be related to specific gene complexes just as the experimental results show.

In consideration of the physiology preceding tumor development, however, one might expect a specific genic difference in a certain system to influence tumor formation in a number of organs of that system. In this light it appears a little surprising that in mice we do not have more evidence that a high incidence of breast tumors is associated with a high incidence of uterine tumors, or ovarian tumors, or tumors of various other endocrine organs. A possible illustration is to be found, however, in the lethal yellow gene that increases the incidence of pulmonary tumors, an effect which it has been suggested may be associated rather directly with its ability to increase body size (Heston, 1942c). It has also been demonstrated that this gene has an influence on the development of mammary tumors, although it is suggested that this action is through the hormonal system (Little, 1934).

The incidence data for the inbred strains also give some indication of the probable number of genetic factors involved in the development of

the different types of tumors. As yet there is no conclusive evidence that any tumor of the mouse is controlled by but a single gene. Instead multiple-factor inheritance appears to be involved in each. Evidence against single-factor inheritance is given by the variation in degree of susceptibility as expressed by the variation in tumor incidence of the different strains, together with the more conclusive data from the hybridization studies discussed later.

The pulmonary-tumor data offer a good example. Strain A has an incidence of around 90 per cent and strains C57 black, C57 brown, and L incidences of less than one per cent. Other strains such as C3H, C and Swiss show various intermediate degrees of susceptibility. Such a situation can be expected only if one is dealing with multiple genetic factors the cumulated effect of which when added to the nongenetic factors fluctuates about a physiologic threshold, or if one is dealing with a multiple allelic series. The latter possibility, however, has not been indicated by the hybridization studies.

In this respect, tumor genetics falls in line with Wright's findings (1934a, b and c) in regard to otocephaly and polydactyly in the guinea pig where the genetic constitution established the incidence of the strain, while differences between individuals within the strain were controlled by nongenetic factors. The situation can be roughly pictured as one in which in certain strains the genetic complex approaches the threshold so nearly that in most individuals the threshold is surpassed by the added effects of variable nongenetic factors, whereas in other strains the genetic constitution is such that fewer individuals receive sufficient weight of nongenetic factors to surpass the threshold. In the case of pulmonary tumors, a very potent nongenetic factor, a carcinogen, can be introduced to cause more of the animals to surpass the threshold and at an earlier age. Unlike polydactyly, otocephaly and other such characters which appear before birth, the nongenetic factors influencing tumor formation can accumulate throughout the life of the animal, a condition which makes the age factor of extreme importance in any analysis.

In the light of this discussion, mention should be made of the use of the term "dominance" in the cancer literature recently discussed by the author (Heston, 1944). The term as it has been used should not be confused with its use in regard to a specific gene which, when present, provides a specific enzyme that produces a character, as opposed to its recessive allele which fails to bring about the production of the enzyme. Slye mated nontumorous mice to mice that later developed tumors without having homozygous strains and found that the F_1 mice tended to be free of the tumor, although the tumor did appear in animals in later generations, and from such results she concluded that tumor inheritance was recessive in

nature. After the inbred strains were developed, it was found that in the case of certain tumors at least, when mice of the high tumor strains were mated to those of the low tumor strains for the most part the F_1 generation was tumorous. On such results it was concluded that these tumors were inherited in a dominant manner. It should be emphasized that in neither case were the specific genes known. One therefore wonders whether the tumors appeared in the F_1 generation because the specific genes involved were dominant to their alleles or because there was such a high accumulation of favorable factors in the high tumor strains that, although the action of certain ones may have been suppressed with dominant alleles in the F_1, the total effect still surpassed the threshold in most cases. The fact that pulmonary tumors appear at a later date in the F_1 generation than in the high-tumor strain suggests this possibility.

In the following sections hybridization studies of the various types of tumors are discussed.

III. Mammary Tumors

After the development of the inbred strains with high and low mammary tumor incidences, the next logical step was the hybridization of these strains. Between 1930 and 1933 members of the staff of the Jackson Memorial Laboratory (Jackson Memorial Laboratory, 1933) made reciprocal crosses between the following strains: strain dba (high tumor) and C57 black (low tumor), strain A (high tumor) and CBA (low tumor), strain dba and *Mus bactrianus* (low tumor), and strain C3H (high tumor) and I (low tumor). In the four experiments 532 F_1 females were produced, the results of which were consistent in showing that the mammary tumor incidence of the F_1 females resembled that of the strain of the mother. This difference persisted into the F_2 generation, as was shown by the results of 1,139 F_2 females from the crosses between dba and C57 black (Murray and Little, 1935a), and later it was also shown that the maternal effect was apparent in the backcross generations (Murray and Little, 1935b).

At about the same time Korteweg (1934, 1936) made similar crosses between strain dba and strain C57 black mice which he had obtained from the Jackson Memorial Laboratory; and although his numbers were not large, the results were in line with those of Little's group.

Since it was considered that the genetic constitution of reciprocal groups of F_1 females resulting from crossing two isogenic strains was the same, it was obvious that this effect was not transmitted on the chromosomes but was some extrachromosomal factor which the female was capable of transmitting to her offspring. Furthermore, it appeared that

the factor was likely transmitted through one of three possible routes: the cytoplasm of the egg, the placenta, or the milk.

Korteweg felt that transmission through the cytoplasm of the egg was the most plausible since transmission through the other two routes indicated to him an infectious agent. Later developments, however, have failed to give any indication that the influence can be transmitted in this manner. Fekete and Little (1942; Little, 1944) undertook the problem of the possible intra-uterine influence with an approach through the reciprocal transplantation of fertilized ova between strains dba and C57 black. They interpreted their results as suggesting an added effect of the intra-uterine environment, but verification is needed from repeat experiments not complicated by the milk. Bittner (1936, 1937a, b and c) undertook the third possibility, the milk, and it was through his foster-nursing work that the factor was revealed, and one of the most attractive fields of cancer research was opened.

While this agent is not a genetic factor, a brief summary of the work with the agent is included because of its relationship to the genetic work. A more extensive review has recently been given by Andervont (1945a).

1. *The Milk Agent*

The presence of the agent in the milk was demonstrated by Bittner's original account (1936), in which he reported that high-tumor strain A females taken from their mothers at an early age and nursed upon low-tumor strain CBA foster mothers gave a lower tumor incidence than that expected for the strain A. These results have been verified repeatedly by subsequent reports of Bittner (1937a, b and c) and also by the work of Andervont (1940b; Andervont and McEleney, 1939), Murray (1941), Fekete and Little (1942), De Ome (1940), van Gulick and Korteweg (1940), Furth *et al* (1942), Barnes and Cole (1941), Dmochowski and Gye (1943), and others. In general it has been noted that when the young females of the high-tumor strains were nursed upon foster mothers of low-tumor strains, the observed tumor incidence was less than that of the high-tumor strain; and when females of the low-tumor strains were nursed upon foster mothers of high-tumor strains, the tumor incidence was raised. This latter difference, however, has not been nearly so dramatic as the reduction in tumor incidence. To obtain maximum reduction in mammary tumors in the high-tumor strain females it has been necessary to remove the young females before they have nursed upon their own mothers. Even in such females, however, a few mammary tumors will appear. In 200 breeding females comprising the first 7 generations of a colony of C3H females which Andervont (Shimkin, Wyman and Andervont, 1946) started from a litter born by Caesarean section and foster nursed upon C57 black females,

7, or 3.5 per cent, have developed mammary tumors. Heston *et al* are finding a similar incidence in a colony of C_3Hb mice started more recently in the same manner. It will be interesting to see whether or not these females which developed mammary tumors had the agent.

In studying dose response Andervont *et al* (1939) found a correlation between tumor incidence and the length of time the young were permitted to remain with their mothers during the first 48 hours of life. These results were later verified by feeding susceptible young known quantities of milk containing the agent by stomach tube (Andervont *et al,* 1942) and by permitting them to drink the milk (Bittner, 1940a). More recent dilution studies by Bittner (1946) have suggested an optimum amount of the agent. Extracts of either lactating mammary glands or spontaneous mammary tumor tissue produced higher tumor incidences when diluted 10^{-3} or 10^{-4} than 10^{-1}. He also obtained a few tumors following injection of a dilution of 10^{-6}.

The agent is not limited to milk, Bittner (1937a, 1939b and c) demonstrated that it is also found in the spleen, thymus, and lactating mammary gland, by producing tumors in susceptible test females into which he implanted pieces of these organs subcutaneously. These results were later verified by Andervont *et al* (1942). Woolley *et al* (1941) demonstrated the agent in the blood where it is apparently associated with the cellular elements according to the work of Bittner (1945). Extracts from mammary tumors contain the agent (Bittner, 1942a; Bryan *et al,* 1942), but by transplanting the tumors into susceptible young and later removing them after considerable growth Andervont (1945c) found that such a procedure was not an efficient way to transmit the agent, for there was no later development of mammary tumors in those females that had received the t ansplant. Bittner (1941a) failed to demonstrate the agent in liver.

The agent has been demonstrated in the milk throughout the period of lactation. Bittner (1941a, 1942b and c, 1943, 1944) found some indication of a higher tumor incidence among females of third and fourth litters than among those of earlier litters, but such variation has not been observed in Andervont's data (1945a). The agent is normally transmitted before the tumor arises.

The agent has been demonstrated in the commonly used high-mammary tumor strains A, C3H, dba, Marsh, and RIII. The three strains A, C3H, and dba have some relationship in their early development suggesting a possible common origin of the agent, but no relationship to the Marsh strain is indicated in the literature or to the RIII strain, which was developed in France. Low-tumor strains such as C57 black and C57 brown develop a few mammary tumors, but thus far there has been no proof of the agent in these tumors. A more systematic search for the agent

in tumors of the low-tumor strains, however, should be made. Kirschbaum *et al* (1946) have reported mammary tumors following painting with methylcholanthrene in mice of certain genetic types which it was assumed did not have the milk agent. It would be of interest to know, however, whether or not the tumors produced had the agent.

Work of Andervont (1941b; Andervont *et al*, 1942) and Bittner (1942c, 1943) indicates that adult mice are more resistant to the agent than are those less than six weeks of age. There is furthermore a definite genetic control over susceptibility, discussed in a later section. Tumors can be produced by subcutaneous injection, intraperitoneal injection, or oral administration of the agent.

In 1942 Bittner (1942a) demonstrated that the agent was filterable through Seitz filters. Bryan *et al* (1942) found that the agent was sedimented, though not completely, by ultracentrifugation at 60,000 × gravity for one hour, and Visscher *et al*, (1942) reported sedimentation at 110,000 × gravity for one hour. Further studies by Kahler and Bryan (1943) suggested a ribose-nucleic acid complex for the agent with a molecular weight of from three to four million for one of the principal active components and at least five million for a faster moving active component. However, similar components were found in milk of mice with low-mammary-tumor incidences.

Bittner (1942a) found that the agent survived when he added 50 per cent glycerine to the extracts of mammary-tumor tissue and kept at refrigerator temperature for 7 and 9 days. Andervont and Bryan (1944) immersed whole mammary tumors in 50 per cent glycerine-saline solution and kept at 8° C. and found that the agent remained active for 7 days but was inactive after 81, 88, and 96 days. Bittner (1941b) reported that the agent remained active in lyophilized mammary-tumor tissue for 6 months but was inactive a year following the lyophilization.

Andervont and Bryan (1944) gave rabbits two weekly injections of saline extract of mammary tumors and also ultracentrifuge pellet material and found that serum obtained 1 month later neutralized fresh mouse mammary tumor extracts *in vitro*. Later Green and Bittner (1946) reported neutralization of mammary tissue centrifugate with rabbit antiserum prepared by the injection of the same type of material.

2. *Genetic Differences in Mammary-tumor Development*

Hybridization studies have shown that despite the importance of the milk agent genetic differences are of even greater basic importance in the development of mammary tumors and in maintaining high incidences through successive generations. In crosses between high-tumor strain A and low-tumor strain C57 black, Bittner (1940b and c) observed that the

tumor incidence of the F_1 females from A mothers, as well as reciprocal F_1 females foster-nursed by A females, was as high as that of the high-tumor strain A. In both cases the incidence approached 100 per cent. The F_1 females from C57 black mothers had a low incidence since they lacked the agent. The incidence of the F_2 females which had received the agent was approximately 75 per cent, while that of the F_2 females which had not received the agent was negligible. Bittner pointed out that these ratios were in accord with the genetic theory that susceptibility to breast cancer is inherited as a single dominant factor.

Later evidence has demonstrated that the genetic picture is far less simple. More than one coat-color gene has been shown to be associated with mammary-tumor development. In the F_2 generation from a cross between high-tumor strain dba and low-tumor strain C57 black, Murray and Little (1935a) observed that the brown segregants had a higher percentage of tumors than did the black segregants. From calculations of age distributions, however, they found that the browns had a better chance to develop tumors and concluded that this might partially or wholly explain the difference. In the same experiment they also found that in the BdF_2 generation the dilute segregants had a higher incidence than did the intense segregants, thus suggesting linkage between mammary-tumor development and this locus. As they pointed out, however, the dBF_2 generation failed to give supporting evidence.

Bittner (1945) recently reported more conclusive evidence of a relationship between mammary-tumor development and the brown locus. In the F_2 generation from the cross of high-tumor strain A × low-tumor strain C57 black, the brown segregants had a higher incidence than did the blacks. The difference again was not great, and he concluded that probably multiple factors were transmitted, one of which was linked with the brown gene.

It was pointed out earlier that the difference between the lethal yellow gene and the non-agouti allele results in a difference in mammary-tumor development (Little, 1934). The presence of the A^y gene causes the tumors to appear earlier although the final incidence among the mice bearing this gene was lower than that of the homozygous non-agouti females.

3. *Paths of Gene Action*

More recent work has been directed toward an attempt to analyze the physiologic complex preceding mammary-tumor development in an effort to determine where the genic differences are and through what mechanisms the genic actions become manifest (Heston, 1946). A summary of the work to date presents a picture such as is given in fig. 1. In

an attempt to visualize the physiologic mechanisms one can consider that at the time of fertilization, a certain complex of genetic factors is established. This complex is passed from cell generation to cell generation throughout the lifetime of the animal, and at various times certain of these genes enter into the physiology of the animal in such manner as to influence the chances that a mammary tumor will appear.

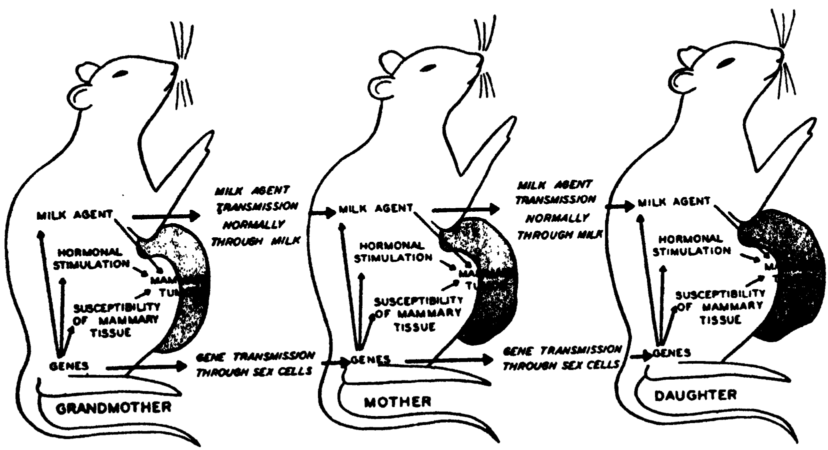


FIG. 1
Gene-mammary tumor relationship

After birth, the mouse begins to nurse upon its mother and receives the milk agent if the mother carries the agent. This agent which, as was noted in a preceding section, has many characteristics of a virus, must be considered to be essential in the development of a high proportion of mammary tumors. The probabilities that the introduction of this agent will result in a mammary tumor, however, is dependent in part upon the type of genes with which the agent finds itself associated. Thus, this physiology in relation to the milk agent constitutes one of the avenues through which genic differences can influence the probabilities for mammary tumor development. Since the agent is present throughout the lifetime of the animal and in many of the tissues throughout the body, we are still a long way from determining just where or when the genic action takes place.

As the mouse matures, the hormonal stimulation enters the picture. There is a vast amount of evidence indicating that proper stimulation is

necessary for the appearance of the tumor. Recent work has shown that this hormonal stimulation is under the influence of genetic factors, and thus the hormonal mechanism constitutes a second avenue through which genic differences can influence the probabilities for tumor formation. In this case one can expect the primary gene action to be in the chemistry of hormone production.

The third avenue through which genic differences in mammary-tumor development can be expressed is through the control of the susceptibility of the mammary-tissue cell. The use of the term "susceptibility" in the past has been somewhat vague, but it is now assuming a more tangible meaning from the evidence that genic differences may vary the reaction of the mammary-tissue cell to the hormonal stimulation and to the milk agent. Since two stimuli are involved, it may be that more than one genic difference is involved and that the actions are effective at different times. In either case, however, the genic action would be expected to be in the physiology of the mammary-tissue cell.

Evidence of the genetic effect on the propagation and transmission of the milk agent was shown in a recent detailed comparison of two groups of backcross females with equal maternal influences but of unlike genetic constitutions (Heston *et al*, 1945). These two groups of backcross females were developed by mating high-tumor strain C3H females to low-tumor strain C57 black males and in turn backcrossing the resulting F_1 hybrid females with C3H males to obtain the susceptible strain backcross, and with C57 black males to obtain the resistant strain backcross females. Since both groups were from a genetically uniform lot of F_1 mothers (F_1 progeny of two isogenic lines) which had the milk agent, it could be considered that both groups had received comparable maternal influences, including the milk agent and possible cytoplasmic or intra-uterine factors; although on the average three-fourths of the chromatin of the one group had been derived from the susceptible strain, while only one-fourth of the chromatin of the other group had. To determine the ability of these backcross females to transmit the agent, the individuals of the two groups were tested with foster-nursed, uniform, test females, the F_1 progeny of C57 black females mated to C3H males.

The importance of the genetic difference was demonstrated by the difference in tumor incidences of these two groups of backcross females. The susceptible strain backcross had an incidence approximately 40 per cent higher than the resistant strain backcross. Since the milk agent and other possible maternal influences were controlled, this difference could be attributed solely to the genetic difference caused by the difference in the genetic constitution of the two paternal strains.

Comparison of the test females of the two groups showed that the

groups differed in their ability to transmit the agent. A higher incidence of mammary tumors was found in those that had nursed upon the susceptible strain backcross females than in those used to test the resistant strain backcross females. This could not be a genetic difference in the test females for they were genetically uniform. The difference indicated a difference in milk agent that the test females received, a difference in turn owing to the genetic difference of the foster mothers.

Further evidence of segregation of genetic factors was apparent in the variation within the two groups of the ability to transmit the agent as ascertained by tumor development in the test females. This was particularly apparent in the resistant strain backcross. In both backcross groups all females that later developed tumors transmitted the agent effectively but those that did not develop tumors showed considerable variation. In certain cases there was no evidence that the agent was transmitted since tumors did not appear in the test females, but other nontumorous backcross females did transmit the agent although on the average not as effectively as did the tumorous females. These results suggested the possibility of two sets of factors, one controlling the propagation of the agent and thus the ability to transmit the agent and the other group the susceptibility to the agent. Unknown nongenetic factors might account for the variation in part.

Other results have been in line with these. When Murray and Little (1939) outcrossed high-tumor strain dba females to low-tumor strain C57 black males and in turn backcrossed the female offspring to the parent strain C57 black males through eight consecutive backcrosses they found that by the eighth backcross generation the milk agent had been eliminated. The explanation offered is that the milk agent was not compatible with the C57 black genetic background. Andervont (1945b) introduced the milk agent into C57 black females and found that whereas those receiving the agent were able to transmit it to susceptible young the female progeny through successive generations could not.

Strain C has appeared to be exceptional in this regard. When Andervont (1941c, 1945b) introduced the milk agent into this strain, it became a high-tumor strain and remained so through successive generations. This indicated that strain C was genetically susceptible to the agent, but in its development had either lost the agent or had not received it.

Considerable emphasis has recently been given to comparisons between the milk agent and so-called plastogenes and plasmagenes (Haddow, 1944; Heston *et al*, 1945). The cytoplasmic factor described by Sonneborn (1945) in Paramecium offers an excellent analogy. Comparison of the gene-milk agent relationship to the relationships between genes and these other agents is of value in that it emphasizes the necessity of portraying the milk

agent as an integral part of the cell, and as an agent that may have arisen by a transformation of some normal cellular component. Furthermore, while purification studies will be extremely valuable, emphasis must be placed on the milk agent not as a separate entity but as one unit among the physiologically coordinated nuclear and cytoplasmic units that comprise the cell.

A genetic difference in hormonal stimulation was demonstrated by crosses between the high-tumor strains A and C3H (Bittner and Huseby, 1946; Bittner *et al*, 1944; Heston and Andervont, 1944). While both are high-tumor strains, they differ in that whereas both the virgin and breeding females of strain C3H have a high tumor incidence only the breeding females of the strain A have, that of the virgins being less than 5 per cent.

Results of reciprocal crosses between the two strains demonstrated that this difference, which one would attribute to a hormonal difference, was fundamentally a genetic difference. The virgin females of both F_1 groups resulting from reciprocal matings had a high tumor incidence. The high incidence in the group with the strain A mothers and C3H fathers indicated that the factors introduced by the sperm were sufficient to raise the incidence from the low incidence of the strain A virgins to a high incidence comparable with that of the strain C3H virgins. We are inclined to consider that any difference controlled by the factors carried in the sperm is a genetic difference.

The question still remained, however, whether the genetic difference was being manifest as a difference in production of the hormonal stimulation or as a difference in the reaction of the mammary-tissue cell to the hormonal stimulation. Certain results suggested a difference in hormonal stimulation. Administration of estrogenic hormones raises the tumor incidence of virgin strain A females (Gardner, 1939; Suntzeff *et al*, 1936, 1941). Furthermore, Jones (1940) showed that there is a correlation between number of pregnancies and tumor incidences.

The problem can best be approached through assay of estrogen output, but small amounts of mouse urine offer difficulties for such determinations. As an alternative, Deringer *et al* (1945) observed the time at which the vagina opened in virgin females of strains A and C3H and their reciprocal F_1 hybrids and followed the estrus cycles through to 150 days of age. She found that after the cycles were started there was little variation between the groups but in the low-tumor strain A virgins, she found that the vagina opened at a significantly later age than in either of the three high-tumor groups. This indicated a difference in hormonal production, but the difference was only approximately 10 days, which was not a great difference; and thus it appeared that some of the difference between the two strains might still be a difference in the reaction of the mammary-

tissue cells unless it could be shown that these early cycles are extremely important ones.

IV. Lung Tumors

The development of lung tumors appears to lend itself to genetic analysis better than that of any other type of tumor. Normally nongenetic differences appear to have less influence in the control of the probabilities that the lung tumor will appear than they have in other types, for example, mammary tumors. Nongenetic factors can have an influence, however. Certain deficient diets decrease the incidence of lung tumors (Larsen and Heston, 1945; Tannenbaum, 1942a and b) and administration of the carcinogenic hydrocarbons have a marked positive effect (Andervont, 1939; Heston, 1940, 1942a; Lynch, 1927, 1940; Shimkin, 1940; Shimkin *et al*, 1946).

In the early work of Lynch (1926) it was found that genetic factors were important and that when high-tumor strains were outcrossed to low-tumor strains a high incidence appeared in the F_1 generation. Her work was also extended to the tumors induced with coal tar and its derivatives (1927, 1940). In this work, she found that genetic differences were again important in regard to the induced tumors not only in controlling incidence, but also in controlling the number of lung nodules per individual.

Bittner (1938; Bittner and Little, 1937) observed ratios suggesting single dominant-factor inheritance of spontaneous lung tumors in F_2 and backcross generations resulting from matings between high-tumor strain A and low-tumor strain C57 black, and backcross ratios of induced tumors reported by Andervont (1937, 1938a) fell in line with this interpretation.

Such a simple interpretation has been broken down by the observations in more recent work, much of which has been facilitated by the use of the hydrocarbons. Although as has generally been observed following the introduction of the hydrocarbon, the incidence is raised, the average latent period is shortened, and the number of tumor nodules per individual is increased, the relationship between the different strains in degree of susceptibility to the induced tumors is the same as for spontaneous tumors (Andervont, 1939; Shimkin, 1940a and b). This fact, together with comparisons of hybrid groups and of effects of specific genes, indicates that the same genetic differences are influential in both types of tumors. Histologically the two types are identical.

Because of the greatly reduced latent period of the induced tumors, it has been much easier to make extensive analysis of the induced type. There has also been the great advantage in that the latent period provides an adequate measure of degree of susceptibility. The higher the susceptibility the shorter the latent period. Furthermore, degree of susceptibility

can also be determined by counting the nodules on the surface of the lung, the more highly susceptible the animal the more nodules are found. The two methods have been found to agree closely.

Analysis of the induced tumors demonstrated that a single-factor interpretation was not adequate in explaining the results obtained. When high-tumor strain A was outcrossed to three low-tumor strains L, N, and W, it was found that the different types of F_1 and backcross hybrids varied in their response to a uniform dose of the carcinogen, indicating a variation between the low-tumor strains in genes influencing this type of tumor (Heston, 1940).

These observations led to an extensive study of crosses between high-tumor strain A and low-tumor strain L (Heston, 1942a). Degree of susceptibility was measured on the basis of latent period and also on the basis of number of tumor nodules, and in both cases the picture presented was typical of that of a multiple-factor, quantitative character.

In order to measure the latent period, sample age groups were taken of the two parent strains and their F_1, F_2 and backcross hybrids, and for each the tumor incidence was determined. The results are illustrated in fig. 2. The highly susceptible strain A had a very short latent period.

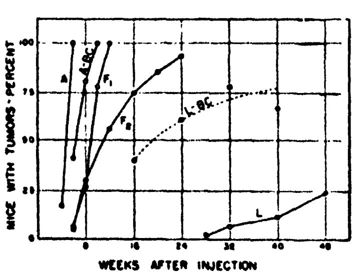


FIG. 2

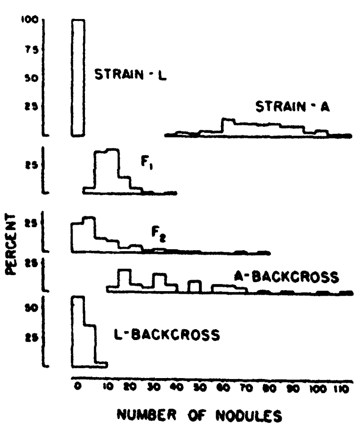


FIG. 3

FIG. 2

Pulmonary tumor incidence in strain A and strain L mice and their F_1, F_2, and backcross hybrids killed at various intervals following the intravenous injection of 0.5 mg. 1,2,5,6-dibenzanthracene. (From Heston, 1942a.)

FIG. 3

Frequency polygons for number of pulmonary tumor nodules in the parent stains A and L, and their F_1, F_2, and backcross hybrids at 16 weeks after the intravenous injection of 0.5 mg. 1,2,5,6-dibenzanthracene. (From Heston, 1942a.)

Tumors were found in 100 per cent of the group being killed at 6 weeks following the injection of the carcinogen. The resistant strain L had a long latent period. The final group was killed at 48 weeks, and at that time only approximately 25 per cent of the animals had tumors. The F_1 generation was intermediate between the two parent strains as was also the F_2 which showed greater variation than the F_1. The backcross generations were intermediate between the F_1 and the respective parent strain.

By measuring degree of susceptibility on the basis of number of nodules, the results illustrated in fig. 3 were obtained. They were strikingly similar to those of the latent-period determinations. Such results indicated multiple genetic factors with cumulative effects.

Linkage studies have yielded positive results (Heston, 1942b and c). It has been shown that susceptibility to induced pulmonary tumors is associated with the linked genes, shaker-2, and waved-2, the flexed-tail gene and the lethal yellow gene. Whether these results were owing to susceptibility genes carried on the chromosome with the marker genes or to an added effect of the identified gene is not clear in all cases. In the case of lethal yellow, however, it was evident that the increase in susceptibility was an added effect of the lethal yellow gene. The effect was observed in the F_1 from the cross between strain A and strain Y bearing the lethal yellow gene which, owing to its lethal effect when homozygous, had remained heterozygous despite the inbreeding of the strain. The positive effect of this gene on lung-tumor development is particularly interesting in respect to its positive effect on body size. The possibility that the other results may have likewise been the added effect of the shaker-2 and of the flexed-tail genes has been considered. In these instances susceptibility was decreased by the introduction of these genes.

These analyses have been extended to spontaneous pulmonary tumors, and the results thus far have been in accord with those of the induced tumors (Heston, 1942b). Degree of susceptibility to spontaneous pulmonary tumors, cannot be measured on the basis of number of nodules, since usually but one nodule is present and very seldom are there more than three or four. Latent period is of value, however, in getting a more accurate measure of degree of susceptibility. When latent period was considered, the F_1 generation had to be considered as intermediate to the parent strains A and L since although the final tumor incidence approached that of strain A, the tumors occurred at an older age. This age difference was also present in Bittner's data but had been disregarded. The backcross of the $F_1 \times$ strain L was intermediate between the F_1 and strain L. Such results, together with the variation in degree of susceptibility of the various isogenic strains referred to earlier, indicated that spontaneous pul-

monary tumors were likewise inherited on a multiple-factor basis. The crucial test is the breeding test of the backcross segregants, an experiment remaining to be done.

In extending the linkage work to spontaneous tumors the lethal yellow gene has been shown to have an effect on the spontaneous pulmonary tumors comparable with that on those induced (Heston and Deringer 1947.). Data on tests for linkage of the other genes to the spontaneous tumors are not yet available.

V. Leukemia

Inbreeding with selection has resulted in certain strains of mice with high incidences of leukemia as opposed to others with low incidences just as has occurred in relation to other types of neoplasia. MacDowell's strain C58 has an incidence of approximately 90 per cent, while that of Furth's strain Ak is around 70 per cent. Strain dba has a relatively high incidence although it has not been definitely determined for all the different sublines. Other strains are resistant. That such strain differences have been developed in this way constitutes evidence that certain genes are favorable to the development of leukemia whereas the presence of others decrease the probability that the condition will arise.

In an extensive genetic study, Cole and Furth (1941) demonstrated that susceptible strain Ak differed from resistant strain Rf by many genes influencing the development of leukemia. In the analysis of the F_1, F_2, F_3 and various backcross generations it was found that the common logarithm of the per cent leukemia was a simple function of the per cent heredity from the high-leukemia stock.

MacDowell and his associates (1945) have recently approached the problem through breeding tests of the genotype of first backcross males. High-leukemia strain C58 was mated to low-leukemia parent strain Storrs-Little and the resultant F_1 hybrids were backcrossed to the low-leukemia parent strain. From the backcross hybrids thus produced, 50 males in which the reassortment of genes was indicated by the distribution of hair color types were in turn backcrossed to the low-leukemia strain females, as a breeding test of the genotypes represented in the backcross males. The incidence of leukemia in these second backcross animals failed to give evidence of simple gene segregation. On the contrary, the distribution of the 50 families approximated that of a normal curve indicating many genotypes among the backcross males.

Genetic analysis of this distribution was complicated, however, by the demonstration of a number of variables which in many cases, by modifying length of life, had an influence on the incidence of leukemia.

These variables were: parturition age of mother, strain of nurse, color and intensity of hair pigment, and sex.

The increasing parturition age of the mother progressively delayed the appearance of leukemia without influencing other causes of death with the result that more and more of the potential leukemics died from other causes and the incidence of leukemia fell as the mother's age increased. Thus the genetic differences were revealed in the progeny of the younger mothers. The oldest mothers virtually eliminated leukemia whatever the genetic tendency was.

In order to rear a large number of second backcross females in a short period of time, females of two strains were used as nurses, the parent resistant strain Storrs-Little and the Bagg albino strain that was also a low-leukemia strain. When compared with the Storrs-Little nurses, the Bagg albino nurses tended to increase the incidence owing principally to the increase in life of the males. Such a difference is basically genetic in regard to the foster nurses but not in regard to the nursed young. It should be emphasized that this evidence in no way indicates a milk agent comparable to that involved in mammary tumor development in mice.

There was some indication of an association of the black coat color gene with leukemia and a much stronger indication that the dilution gene also had an effect. It was interesting that the dilution gene coming from the low-leukemia strain acted as a plus modifier for leukemia, a condition similar to that demonstrated by the author for the effect of the lethal yellow gene on induced pulmonary tumors (Heston, 1942c) and by Russell (1942) for the effect of one of the chromosomes in *Drosophila melanogaster* on a melanoma in that species.

The tendency for females to have a higher incidence of leukemia than males as found by Cole and Furth was also seen in this work of MacDowell although the difference was not highly significant. Explanation offered for the difference, however, was that femaleness lengthened life by resisting certain non-leukemic causes of death, thereby increasing for females the opportunity for potential leukemics to appear.

Comparison of spontaneous leukemia with induced leukemia has proven interesting in that the genetic factors involved in induced leukemia do not necessarily coincide with those involved in spontaneous leukemia. Strain dba in which the spontaneous condition occurs rather frequently is susceptible to leukemia induced by 9:10 dimethyl-1:2 benzanthracene as shown by the work of Law (1941) and also to that induced by methylcholanthrene and benzpyrene as shown by Mider and Morton (1939). With the same technique, however, Engelbreth-Holm and Lefèvre (1941) obtained somewhat fewer cases of leukemia in the same strain, but the incidence of spontaneous leukemia is less in their subline. Kirschbaum and Kaplan

(1941), however, compared a number of strains as to the incidence of spontaneous leukemia, leukemia induced by radiation, and leukemia induced by methylcholanthrene, and found no correlation between the susceptibility to any two of the different types. This would indicate that different physiologic mechanisms were involved for the three different types.

VI. Subcutaneous Tumors

Practically all the genetic work on subcutaneous sarcomas has been done with induced tumors since spontaneous tumors of this type are rare in the inbred strains of mice. It is, however, a frequently occurring tumor following the subcutaneous injection of the carcinogenic hydrocarbons in many of the strains.

Some of the most extensive work with this type of tumor has been that of Andervont (1933a, b; 1940a). He demonstrated strain differences in susceptibility to this type of tumor, differences that can be safely assumed to have been primarily genetic. When he mated strain C3H with strain I and strain Y mice he observed that the susceptibility of the first generation hybrids was intermediate to that of both parents.

More recently, Burdette (1943) has reported on tumors induced by methylcholanthrene in the progeny of strains C3H and JK mice. He found C3H more highly susceptible than JK and F_1 hybrids intermediate, and from these results he pointed out that if the difference is genetic and dominance is complete one must conclude that the strains differ by two or more genes and that not all are dominant and not all are recessive. In this analysis, however, although subcutaneous sarcoma was the predominant type of tumor, the data also included a few induced epidermoid carcinomas and rhabdomyosarcomas. As with Andervont's results there was no evidence of an extrachromosomal influence and no evidence of sex linkage.

VII. Gastric Tumors

Because of the high incidence of gastric carcinoma in man, there has been a great demand for experimental work on this neoplasm. Unfortunately, however, a suitable experimental animal has not been available. Strain I mice have a high incidence of adenoma of the glandular stomach, but since the lesion is benign this strain has not satisfied the need. Comparable adenomatous lesions are occassionally found also in other strains and in hybrid mice. Recently Strong (1945) has reported his development of a strain of mice with a high incidence of gastric carcinoma through genetic procedures aided by routine injection of methylcholanthrene. Should this strain prove to meet the wide-spread need for a strain that developed

carcinoma of the glandular stomach it would indeed be a great contribution. All reports on the strain have been from Dr. Strong's laboratory, and thus especially since this is such an important project, reports from other workers in other laboratories confirming the diagnosis as carcinoma distinct from the more commonly occurring adenoma will be of interest.

Strong mated strain CBA with strain N and inbred the CBAN hybrids to the ninth generation. At this time the CBAN stock was outcrossed to strain JK producing what he termed the NH stock. At the fourth generation of inbreeding of this stock the routine procedure of injecting all breeding mice with 1 mg. of methylcholanthrene subcutaneously at 60 days of age was established. This injected line was then designated as NHO. He reported gastric carcinoma in the mice of this strain following the injections and subsequently as the treatment was continued generation after generation. He now reports that lines in which the treatment has been discontinued also develop gastric cancer and pass the tendency for the cancer to their offspring. According to his later reports the incidence is increasing as he is continuing his inbreeding to stabilize the strain.

In explanation Strong has proposed that the action of the methylcholanthrene on the production of the malignant lesions may be of a twofold nature: (1) the conversion of the gastric mucosa into cancer, and (2) the production of a germinal mutation in a genetic entity which in some manner or other underlies certain morphologic or physiologic characteristics of gastric mucosa. If alteration of the germ plasm with methylcholanthrene is confirmed a remarkable finding will have been established. Since a genetically heterogenous stock was employed, however, the possibility that the high incidence of the lesion resulted from mere selection of genes cannot be ruled out. It would be interesting if similar results could be obtained using an isogenic strain, or if a high gastric cancer line could be selected without methylcholanthrene.

VIII TUMORS OF THE FOWL

Of primary recent interest among tumors of the fowl has been the complex of lymphomatosis in chickens, which apparently has a number of manifestations including visceral, neural, and ocular lymphomatosis and osteopetrosis. The economic importance of this disorder has demanded a concerted attack by the group at the U.S. Regional Poultry Research Laboratory at East Lansing, Michigan, and cooperative experiment stations, (Waters, 1945a and b; Waters and Prickett, 1944; U.S. Regional Poultry Research Laboratory Report, 1945). The disorder is also being studied in Gowen's laboratory.

That genetic factors are involved in controlling susceptibility has been demonstrated by the ability to produce divergent lines through

inbreeding and selecting. Age at which the condition arises has been a valuable criterion on which to base selection. Progress in this work is necessarily slow, but results thus far suggest that breeding procedures will prove to be an effective means of reducing the incidence of the disease.

An agent or agents for the complex are evident. Results indicate that transmission can be through the egg as well as by contact. Transmission is also readily attained with a cellular inoculum which usually results in a tumor at the site of inoculation with metastases to other organs. The agent is filterable. Injection of the cell-free extracts produced visceral lymphomatosis and osteopetrosis without inducing a tumor at the site of inoculation.

The fact that lymphomatosis is transmissible by a contagious agent has raised the question in the minds of some as to whether or not this malignant disease should be considered as a true neoplasm. Mammary tumor of the mouse was described as a neoplasm before it was found to be associated with an agent which is contagious in that it is transmitted from the mother to the young through the milk.

IX. Tumors of Fishes

Melanomas in fishes offer an excellent example of a genetically controlled tumor. Work of Gordon (1941) and others has shown that macromelanophores, inherited as a sex-linked dominant gene *Sp* evoke melanotic tumors under the influence of certain multiple modifying genes of which *A* and *B* are suggested. When the spotted platyfish (*Platypoecilus maculatus*) is outcrossed to the albino swordtail (*Xiphophorus hellerii*) or to a number of other non-spotted species of platyfish, melanomas appear in the hybrids, owing to the influence of the modifying factors introduced by the outcross, on the macromelanophore cell. With this genetic combination the macromelanophores undergo hyperplasia and later develop into invasive tumors, in which cells typical of mammalian melanomas, the melanoblasts, the fibrocytes, and macrophages, appear.

Micromelanophores which are inherited as an autosomal dominant *St* occur in these crosses. Their presence seems to intensify the melanotic tumor but is not essential since the tumors are found in hybrids in which the micromelanophores are not shown. Colorless tumors have been observed apparently under the influence of the albino factor *ii*.

The tumor is of particular interest in that it appears to offer an approach to the study of the malignant change. The way is open for the identification of the specific genes affecting the malignant transformation. If the macromelanophore can be established definitely as the cell of origin of the tumor, the material will offer an excellent opportunity for cytochemical study on the comparison of the normal with the malignant cell,

especially since both the macromelanophores and the melanomas can be grown in tissue culture. Here the specific action of these modifying genes might be revealed.

X. Tumors of Drosophila

The melanotic tumors of Drosophila have provided an investigative field of interest, not in that they can be compared directly to mammalian cancer, but in that they do represent abnormal growth in an organism in which the gene complex is better understood and controlled than in any other organism.

These tumors are benign growths which appear in the later part of the larval life as nodes or morulae of small cells coated with a layer of melanin. They are found in various regions of the body. Growth is rapid and the number of tumors increases until pupation, at which time growth ceases abruptly with the break-down of the tissues. It is probably this factor that prevents the growths from killing the host. Tumors from late pupae and adults contain very few cells. One tumor l (1)7 was described by Bridges (1916) and Stark and Bridges (1926) as malignant, but Russell later showed that death of the host was owing to starvation because of a gut abnormality occurring in the strain. The tumors can be readily transplanted.

Bridges showed that the tumor *l (1)7* was inherited as a sex-linked recessive, but the inheritance of all the others analyzed has been multiple-factor. In this respect, they are comparable with tumors in mammals, but the Drosophila species has offered a better opportunity for locating the factors involved and determining the weight of each.

One of the tumors studied by Stark and Bridges occurred with an incidence of 100 per cent in the strain they developed. Genes on the second and third chromosomes, principally one on the second chromosome near speck and one on the third at locus 25, contributed 80 per cent of this incidence while 20 per cent was contributed by genes on the X and fourth chromosome. Wilson (1924) described one as occurring in 20 per cent of the larvae of an apparently homozygous strain. Multiple factors were involved, important genes being located in the right half of the second and right end of the third chromosomes. In another with an incidence of 100 per cent in a selected strain, linkage tests identified important factors on the second and third chromosomes with modifiers on the X and fourth.

More recently Russell (1940, 1942) has described one in which non-genetic factors as well as genetic factors were influential since only a 41-per cent incidence was attained in her homozygous line. The nongenetic factors were not identified, although scarcity of food or other crowding conditions and temperature were suggested. They could not, however,

account for all the nongenetic variation. She demonstrated that a gene or genes on the second chromosome were essential for any tumors in the stock; that the effect of the X chromosome was to increase the incidence, but that the net effect of the third was to decrease the incidence.

In regard to future study, it would seem that this organism in which the genes are so well controlled and identified might offer an approach to a knowledge of the physiologic links between the actions of the specific genes and the resultant uncontrolled cell growth.

XI REFERENCES

Andervont, H. B., *Publ. Hlth. Rep. Wash.* **52,** 304–315 (1937).

Publ. Hlth Rep. Wash. **53,** 232–237 (1938a).

Publ. Hlth Rep. Wash. **53,** 1647–1665 (1938b).

Publ. Hlth Rep. Wash. **54,** 1512–1519 (1939).

J. Nat. Cancer Inst. **1,** 135–145 (1940a).

J. Nat. Cancer Inst. **1,** 147–153 (1940b).

J. Nat. Cancer Inst. **1,** 737–744 (1941a).

J. Nat. Cancer Inst. **2,** 13–16 (1941b).

J. Nat. Cancer Inst. **2,** 307–308 (1941c).

J. Nat. Cancer Inst. **3,** 359–365 (1943).

Publ. Amer. Assoc. Advancement Sci. **22,** 123–139 (1945a).

J. Nat. Cancer Inst. **5,** 383–390 (1945b).

J. Nat. Cancer Inst. **5,** 397–401 (1945c).

Andervont, H. B. and Bryan, W. R., *J. Nat. Cancer Inst.* **5,** 143–149 (1944).

Andervont, H. B., and McEleney, W. J., *Publ. Hlth Rep. Wash.* **54,** 1597–1603 (1939).

Andervont, H. B., Shimkin, M. B., and Bryan, W. R., *J. Nat. Cancer Inst.* **3,** 309–318 (1942).

Andervont, H. B., and Stewart, H. L., *Science* **86,** 566–567 (1937).

Barnes, W. A., and Cole, R. K., *Cancer Research* **1,** 99–101 (1941).

Beadle, G. W., *Chem. Rev.* **37,** 15–96 (1945).

Bittner, J. J., *Amer. J. Cancer* **25,** 614–620 (1935).

Science **84,** 162 (1936).

J. Hered. **28,** 363–365 (1937a).

Amer. J. clin. Path. **7,** 430–435 (1937b).

Amer. J. Cancer **30,** 530–538 (1937c).

Publ. Hlth Rep. Wash. **53,** 2197–2202 (1938).

Publ. Hlth Rep. Wash. **54,** 380–392 (1939a).

Publ. Hlth Rep. Wash. **54,** 1827–1831 (1939b).

Amer. Jour. Cancer **36,** 44–50 (1939c).

Proc. Soc. exp. Biol., N. Y. **45,** 805–810 (1940a).

J. Nat. Cancer Inst. **1,** 155–168 (1940b).

Amer. J. Cancer **39,** 104–113 (1940c).

Trans. Coll. Phys. Philad. **9,** 129–143 (1941a).

Science **93,** 527 (1941b).

Science **95,** 462 (1942a).

Cancer Research **2,** 540–545 (1942b).

Cancer Research **2,** 710–721 (1942c).

Cancer Research **3,** 441–447 (1943).

Bittner, J. J. (*continued*)
Cancer Research **4,** 159–167 (1944).
Gibson Island Symposium on Cancer, 1944, *Pub. Amer. Assoc. Advancement Sci.* (1945).
Cancer Research **6,** 493 (1946).
Bittner, J. J., and Huseby, R. A., *Cancer Research* **6,** 235–239 (1946).
Bittner, J. J., Huseby, R. A., Visscher, M. B., Ball, Z. B., and Smith, F., *Science* **99,** 83–85 (1944).
Bittner, J. J., and Little, C. C., *J. Hered.* **28,** 117–121 (1937).
Bridges, C. B., *Genetics* **1,** 1–52 (1916).
Bryan, W. R., Kahler, H., Shimkin, M. B., and Andervont, H. B., *J. Nat. Cancer Inst.* **2,** 451–455 (1942).
Bryan, W. R., Klinck, G. H., and Wolfe, J. M., *Amer. J. Cancer* **33,** 370–388 (1938).
Burdette, W. J., *Cancer Research* **3,** 318–320 (1943).
Cloudman, A. M., Bittner, J. J., and Little, C. C., *Occ. Publ. Amer. Assoc. Advancement Sci.* **4,** 37–41 (1937).
Cole, R. K., and Furth, J., *Cancer Research* **1,** 957–965 (1941).
DeOme, K. B., *Amer. J. Cancer* **40,** 231–234 (1940).
Deringer, M. K., Heston, W. E., and Andervont, H. B., *J. Nat. Cancer Inst.* **5,** 403–405 (1945).
Dmochowski, L., and Gye, W. E., *Brit. J. exp. Path.* **24,** 223–226 (1943).
Engelbreth-Holm, J., and Lefèvre, H., *Cancer Research* **1,** 102–108 (1941).
Fekete, E., and Little, C. C., *Cancer Research* **2,** 525–530 (1942).
Furth J., Cole, R. K., and Boon, M. C., *Cancer Research* **2,** 280–283 (1942).
Gardner, W. U., *Arch. Path. Lab. Med.* **27,** 138–170 (1939).
Gordon, M., *Cancer Research* **1,** 656–659 (1941).
Green, R. G., and Bittner, J. J., *Cancer Research* **6,** 499 (1946).
van Gulik, P. J., and Korteweg, R., *Proc. Nederland. Akad. van Wetenschappen* **43,** 891–900 (1940).
Haddow, A., *Nature, Lond.* **154,** 194–199 (1944).
Heston, W. E., *J. Nat. Cancer Inst.* **1,** 105–111 (1940).
J. Nat. Cancer Inst. **2,** 127–132 (1941).
J. Nat. Cancer Inst. **3,** 69–78 (1942a).
J. Nat. Cancer Inst. **3,** 79–82 (1942b).
J. Nat. Cancer Inst. **3,** 303–308 (1942c).
J. Nat. Cancer Inst. **5,** 161–171 (1944).
Publ. Amer. Assoc. Advancement Sci. **22,** 55–84 (1945).
J. Nat. Cancer Inst. **7,** 79–85 (1946).
Heston, W. E., and Andervont, H. B., *J. Nat. Cancer Inst.* **4,** 403–407 (1944).
Heston, W. E., and Deringer, M. K., *J. Nat. Cancer Inst.* **7,** 463–465 (1947).
Heston, W. E., Deringer, M. K., and Andervont, H. B., *J. Nat. Cancer Inst.* **5,** 289–307 (1945).
Jackson Memorial Laboratory, Staff of the Roscoe B., *Science* **78,** 465–466 (1933).
Jacobsen, O., Heredity in breast cancer. H. K. Lewis and Co. Ltd., London (1946).
Jones, E. E., *Amer. J. Cancer* **39,** 94–99 (1940).
Kahler, H., and Bryan, W. R., *J. Nat. Cancer Inst.* **4,** 37–46 (1943).
Kirschbaum, A., and Kaplan, H. S., *Science* **100,** 360–361 (1944).
Kirschbaum, A., Williams, W. L., and Bittner, J. J., *Cancer Research* **6,** 484 (1946).
Korteweg, R., *Ned. Tijdschr. Geneesk.* **78,** 240–245 (1934).
Korteweg, R., *Genetica* **18,** 350–371 (1936).
Larsen, C. D., and Heston, W. E., *J. Nat. Cancer Inst.* **6,** 31–40 (1945).

Law, L. W., *Cancer Research* **1,** 564–571 (1941).
Little, C. C., *J. exp. Med.* **59,** 229–250 (1934).
In, Staff of the Roscoe B. Jackson Memorial Laboratory, Biology of the Laboratory Mouse, G. D. Snell, Editor, Ch. 6. Blakiston Co., Phila. (1941).
J. Amer. med. Assoc. **125,** 93–97 (1944).
Little, C. C., and Gorer, P. A., In, Grüneberg, H.: The Genetics of the Mouse. Appendix I. Cambridge University Press (1943).
Little, C. C., Murray, W. S., and Cloudman, A. M., *Amer. J. Cancer* **36,** 431–450 (1939).
Lynch, C. J., *J. exp. Med.* **43,** 339–355 (1926).
J. exp. Med. **46,** 917–933 (1927).
Proc. Soc. exp. Biol., N. Y. **43,** 186–189 (1940).
MacDowell, E. C., Potter, J. S., and Taylor, M. J., *Cancer Research* **5,** 65–83 (1945).
MacDowell, E. C., and Richter, M. N., *Arch. Path. Lab. Med.* **20,** 709–724 (1935).
Mider, G. B., and Morton, J. J., *Amer. J. Cancer* **37,** 355–363 (1939).
Murray, W. S., *Amer. J. Cancer* **20,** 573–593 (1934).
Cancer Research **1,** 790–792 (1941).
Murray, W. S. and Hoffman, J. C., *Cancer Research* **1,** 298–307 (1941).
Murray, W. S., and Little, C. C., *Genetics* **20,** 466–496 (1935a).
Science **82,** 228–230 (1935b).
Amer. J. Cancer **37,** 536–552 (1939).
Russell, E. S., *J. exp. Zool.* **84,** 363–385 (1940).
Genetics **27,** 612–618 (1942).
Shimkin, M. B., *Arch. Path. Lab. Med.* **29,** 229–238 (1940a).
Arch. Path. Lab. Med. **29,** 239–255 (1940b).
Shimkin, M. B., Wyman, R. S., and Andervont, H. B., *J. Nat. Cancer Inst.* **7,** 77–78 (1946).
Snell, G. D., Mouse Genetics News. No. 1. Issued by Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me. (1941).
Sonneborn, T. M., *Amer. Nat.* **79,** 318–339 (1945).
Stark, M. B., and Bridges, C. B., *Genetics* **11,** 249–266 (1926).
Strong, L. C., *Cancer Research* **2,** 531–538 (1942).
J. Nat. Cancer Inst. **5,** 339–356 (1945).
Suntzeff, V., Burns, E. L., Moskop, M., and Loeb, L., *Amer. J. Cancer* **27,** 229–245 (1936).
Suntzeff, V., Kirtz, M. M., Blumenthal, H. T., and Loeb, L., *Cancer Research* **1,** 446–456 (1941).
Tannenbaum, A., *Cancer Research* **2,** 460–467 (1942a).
Cancer Research **2,** 468–475 (1942b).
U.S. Regional Poultry Research Laboratory, East Lansing, Mich. Sixth Annual Report, July 1, 1944 to June 30, 1945.
Visscher, M. B., Green, R. G., and Bittner, J. J., *Proc. Soc. exp. Biol., N. Y.* **49,** 94–96 (1942).
Waters, N. F., *Poult. Sci.* **24,** 226–233 (1945a).
Poult. Sci. **24,** 259–269 (1945b).
Waters, N. F., and Prickett, C. O., *Poult. Sci.* **23,** 321–333 (1944).
Wilson, I. T., *Genetics* **9,** 343–362 (1924).
Woolley, G. W., Law, L. W., and Little, C. C., *Cancer Research* **1,** 955–956 (1941).
Woolley, G. W., and Little, C. C., *Cancer Research* **5,** 203–219 (1945a).
Woolley, G. W., and Little, C. C., *Cancer Research* **5,** 506–509 (1945b).
Wright, S., *Genetics* **19,** 471–505 (1934a).
Genetics **19,** 506–536 (1934b).
Genetics **19,** 537–551 (1934c).

Cytology of Coccids (Coccoïdea–Homoptera)

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CONTENTS

I. INTRODUCTION

The present status of coccid taxonomy reflects the fact that probably not more than 10 to 20 per cent of the existing forms are as yet known

(Ferris, 1937). And of these, as Ferris has recently emphasized, our knowledge is still lamentably incomplete; fundamental aspects of structure remain unexplored; males and early nymphal stages which frequently display significant evolutionary diversity are in many groups still unstudied and often still unknown. In the approximately 2750 species now recognized, we are confronted with an immense assemblage of groups representing many lines of evolution in morphology, behavior, life cycles, distribution and general ecological relations but only here and there can the interrelationships of these lines be clearly established. Classification into categories higher than genera is still at many points tentative (Morrison, 1928; Ferris, 1937); hardly are tribes and subfamilies set up but a new species is found to invalidate the proposed criteria of separation. This stage in the emergence of an evolutionarily significant taxonomy is one of extraordinary fascination, and the comparative neglect which the group has suffered is the more surprising in that its study has already disclosed problems of great general biological interest.

Cytologically the record presents even greater lacunae; only the merest beginning has been made. And here again, not one of some forty species sampled has failed to yield cytological data of significance for — and usually challenging to — our concepts of cytological mechanism. So marked is the latter attribute that a renowned cytogeneticist recently greeted the discovery of a new and anomalous type of mitotic mechanism in his own heretofore orthodox material as "cockeyed as a coccid." The wide range in diverse and often unique cytological mechanism among coccids may be suggested by the fact that within the few groups thus far studied are encountered both diploid and haploid parthenogenesis, functional hermaphroditism, unique mitotic figures with intranuclear, or monopolar, or terminally divergent spindles, and spindles with all the chromosomes lined up in chain along the interpolar axis; segregation occurs without prior synapsis; chromosomes migrate out of the nucleus; and polar bodies take part in development. These are in no sense abnormalities, but well established, successfully functioning mechanisms which must be taken into account in the formulation of cytogenetic theory. As an antidote to premature generalization it is salutary to recognize the flexibility of the cytological mechanism involved.

This review attempts to summarize and re-evaluate in terms of present cytological concepts the available data on coccid cytology now scattered in detailed studies of individual species. The evolution of cytological mechanisms within the group and the bearing of the data on coccid systematics will be briefly considered, although as already indicated neither taxonomical nor cytological study has yet reached a stage justifying final conclusions in this area.

Only nuclear or chromosomal cytology is here considered. That coccids offer equally interesting material for cyto-histological analysis is already evident in such studies as Pollister's (1937) on wax glands or Parr's (1940) on inter-relations of insect and host plant. The cytology of symbiosis and the complex mutual adaptations of coccid and symbiont constitute a field in itself and one which should eventually prove of critical value in elucidating evolutionary relationships.

II. The Coccid Chromosome

A. *Somatic Mitosis*

The Coccoïdea in general are difficult material for cytological analysis. Of those thus far sampled the primitive subfamily Monophlebinae (Table 1, page 130) affords a delightful exception; here chromosome number is low and size relatively large. And even in the other groups it is probable that modern methods will permit analysis of cases considered unworkable twenty years ago.

In structure and behavior the salient features of the coccid chromosome stem directly from the diffuse nature of the kinetochore. The kinetic functions are a property of the chromosome as a whole, and are not, as in most organisms, restricted to a specialized zone or organelle. Structurally this is evident in the absence at all stages of the chromosome cycle of any primary constriction or localized kinetochore. In mitosis the diffuse kinetochore results in distinctive types of orientation at metaphase, mode of formation of chromosomal fibers, and anaphase disjunction, and the potentiality — realized to varying degrees in different tissues and species — of complete spatial separation of the constituent chromonematal strands of the chromosome.

In basic pattern the mitotic cycle, as conditioned by the diffuse kinetochore, is uniform in somatic cells throughout the group. The following resumé is based on somatic mitosis in Nautococcus (Hughes-Schrader, 1942) and the single spermatocyte division — somatic in type — of the haploid Steatococcus (Hughes-Schrader and Ris, 1941). The prophase chromosome emerges from the resting condition as an irregularly twisted thread. As coiling proceeds its two constituent chromatids undergo progressive separation (fig. 13, *a–c*). By late prophase the dual nature of each chromatid coil becomes in turn apparent as the constituent quarter chromatid's coils similarly slide apart (fig. 13, *d*). At metaphase the chromosome thus comprises four parallel coils, separated throughout their length (fig. 1, *b*, and 13, *f*). The degree of this separation varies but is frequently complete, since the diffuse kinetochore offers no such block to the separation of chromonematal strands as is presented by the localized kinetochore body.

TABLE 1

Taxonomic Position and Chromosome Number of Cytologically Known Coccids

Taxonomic Position	Chrom. No. ♀	Chrom. No. ♂	Reference
Section MARGAROÏDAE			
Family Margarodidae			
Subfamily Xyloccinae			
Tribe Matsucoccini			
Matsucoccus gallicola Morr.	40	34	Hughes-Schrader, vide infra, p. 190
Subfamily Coelostomidiinae			
Tribe Coelostomidiini			
Marchalina hellenica (Genn.)	18		Hovasse, 1930
Nautococcus schraderae Vayss.	6	5	Hughes-Schrader, 1942
Subfamily Monophlebinae			
Tribe Llaveiini			
Llaveia bouvari (Sign)	6	5	Hughes-Schrader, 1931
Llaveia oaxacoensis Morr.	6	5	Hughes-Schrader, unpub.
Llaveiella taenechina Morr.	6	5	Hughes-Schrader, 1940
Protortonia primitiva (Towns.)	6	5	Schrader, 1931
Tribe Iceryini			
Group 1.			
Gueriniella serratulae (Fabr.)			Vayssiere, 1926
Group 2.			
Crypticerya rosae (Riley and Howard)	4	2	Hughes-Schrader, 1930
Icerya purchasi Mask.	4	2	Hughes-Schrader, 1927, Schrader and Hughes-Schrader, 1926
Icerya montserratensis Riley and Howard	4	2	Hughes-Schrader, 1930
Icerya littoralis Ckll.	4	2	Hughes-Schrader, 1930
Icerya similis Morr.	4	2	Hughes-Schrader, unpub.
Steatococcus tuberculatus Morr.	4	2	Hughes-Schrader and Ris, 1941
Echinicerya anomala Morr.	4	2	Hughes-Schrader, 1930
Family Ortheziidae			
Section LECANOÏDAE			
Family Phenacoleachidae			
Family Eriococcidae			
Subfamily Pseudococcinae			
Puto sp	14	13	Hughes-Schrader, 1944
Pseudococcus citri (Risso.)	10	10	Schrader, 1923
Pseudococcus nipae (Mask.)	10	10	Schrader, 1921, 1923
Pseudococcus maritimus (Ehrh.)	10	10	Schrader, 1923
Pseudococcus adonidum (Linn.)			Walczuch, 1932
Pseudococcus (=Trionymus) *diminutus* (Leon)			Walczuch, 1932
Phenacoccus acericola King	12	12	Hughes-Schrader, 1935
Phenacoccus gossypii Towns. and Ckll.	12	12	Hughes-Schrader, unpub.
Subfamily Eriococcinae			
Gossyparia spuria (Modeer)	28	28	Schrader, 1929
Family Lacciferidae			
Family Stictococcidae			
Family Lecaniidae			
Subfamily Lecaniinae			
Lecanium hesperidum Burm. (=Coccus hesperidum L.)	14	14	Thomsen, 1927
Lecanium hemisphaericum Targ. (=Saissetia hemisphaerica Targ.)	16	16	Thomsen, 1927: Suomalainen, 1940
Lecanium coryli (Linn.)	18		Thomsen, 1929
Lecanium corni Bouche	16		Thomsen, 1929
Family Asterolecaniidae			
Family Dactylopiidae			

TABLE 1 (*Continued*)

Taxonomic Position	Chrom. No. ♀	Chrom. No. ♂	Reference
Dactylopius sp.	10	10	Hughes-Schrader, unpub.
Family Apiomorphidae			
Family Cylindrococcidae			
Family Conchaspidae			
Section DIASPIDOÏDAE			
Family Diaspididae			
Subfamily Diaspidinae			
Tribe Aspidiotini			
Aspidiotus hederae (Vallot)	8		Schrader, 1929
Aonidiella auranti (Mask.)	8*		Dickson, 1932

Metaphase orientation involves the entire chromosome which becomes polarized within the spindle body in such a way that the plane of conjunction between chromatids coincides with the equatorial plane of the spindle.

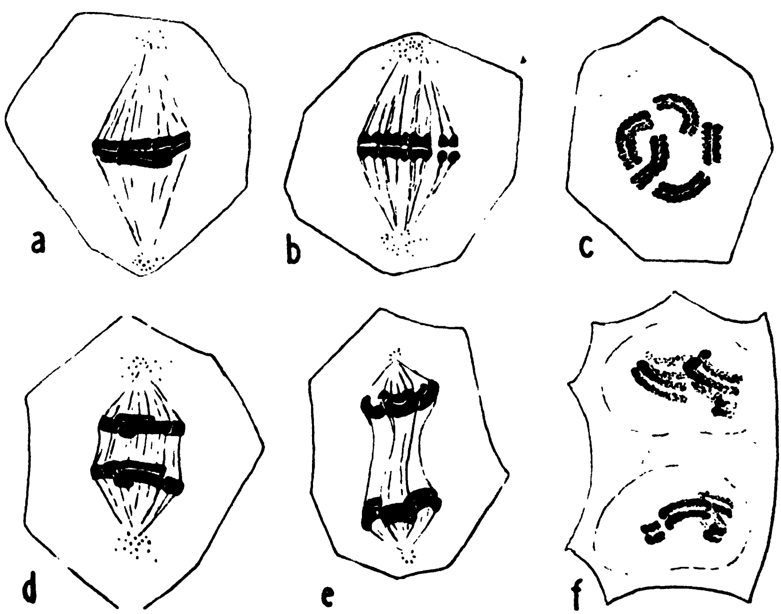


FIG. 1

Somatic mitosis in Nautococcus. *a*, lateral aspect metaphase, chromosomes viewed from side; *b*, same, one chromosome in end view; *c*, same, polar aspect; separation of half chromatids clear; *d*, *e*, anaphasic disjunction; *f*, telophase. From Hughes-Schrader (1942).

A sheet of chromosomal fibers forms from the poleward surface of each half chromatid along its entire length, and these fibers converge to a focus at the pole (fig. 1, *a*, and 13, *e*).

* Counts made in embryos of unknown sex.

Anaphasic disjunction, again conditioned by the diffuse kinetochore is parallel (fig. 1, *d*, and 13, *g*); each chromatid moves toward the pole with its long axis at right angles to that of the spindle. Only at late anaphase do the ends of the chromosomes bend toward the pole (fig. 13, *h*), a movement which Ris (1942) ascribes to a proportional shortening of all chromosomal fibers. The structure of the spindle varies in different somatic tissues. Centrioles and astral rays are in general characteristic of cleavage and become progressively less developed in later stages. Interzonal connections are present; continuous fibers have not been demonstrated.

B. *The Diffuse Kinetochore*

The observational evidence just reviewed defines the diffuse kinetochore in terms of its effect on chromosome structure and behavior. Experimental evidence confirms the observational. If the kinetic functions are properties of the whole chromosome, all fragments of a chromosome should be able to perpetuate themselves mitotically. X-ray induced fragments of the chromosomes of *Steatococcus tuberculatus* (Hughes-Schrader and Ris, 1941) do indeed possess mitotic potency and are capable of perpetuating themselves through many cell generations. Each fragment, irrespective of size, behaves like a normal unbroken chromosome. Prophase coiling and the separation of daughter coils is normal; each fragment orients as a whole at metaphase; chromosomal fibers are produced along the poleward face of each chromatid; and anaphasic disjunction is parallel (fig. 2, *d* to *g*). (This applies to chromosome fragments in eggs and embryos. In irradiated spermatocytes, chromosome and chromatid fragments rarely remain free but undergo extensive fusion. Compare McClintock, 1941.)

Of the structural basis of the diffuse kinetochore nothing is yet known. To assume that it is compound or multiple in nature is unjustified so long as no repetition of structural units can be demonstrated. To suggest that it is compounded of submicroscopic units dispersed over the entire length of the chromosome is to say no more than that it is diffuse. The chromosomal fibers produced by it are indeed multiple, but the same is true of the ordinary kinetochore (Schrader, 1935) and harmonizes as well with an origin of the latter by restriction and subsequent structural differentiation, as with an origin of the former by multiplication. For the present the chromosome with diffuse kinetochore may best be defined as one without localization of kinetic function in a differentiated organelle or restricted zone. In such a chromosome one may speak of the kinetic functions as diffused over the entire chromosome and, for brevity, of a diffuse kinetochore.

Although outside the scope of this review it should be noted that the diffuse kinetochore is not restricted to coccids but is found throughout

Homoptera and Heteroptera. An even wider distribution is probable. Thus Federley (1945) has demonstrated the absence of a restricted localized kinetochore in certain Lepidoptera; and the parallel anaphasic disjunction

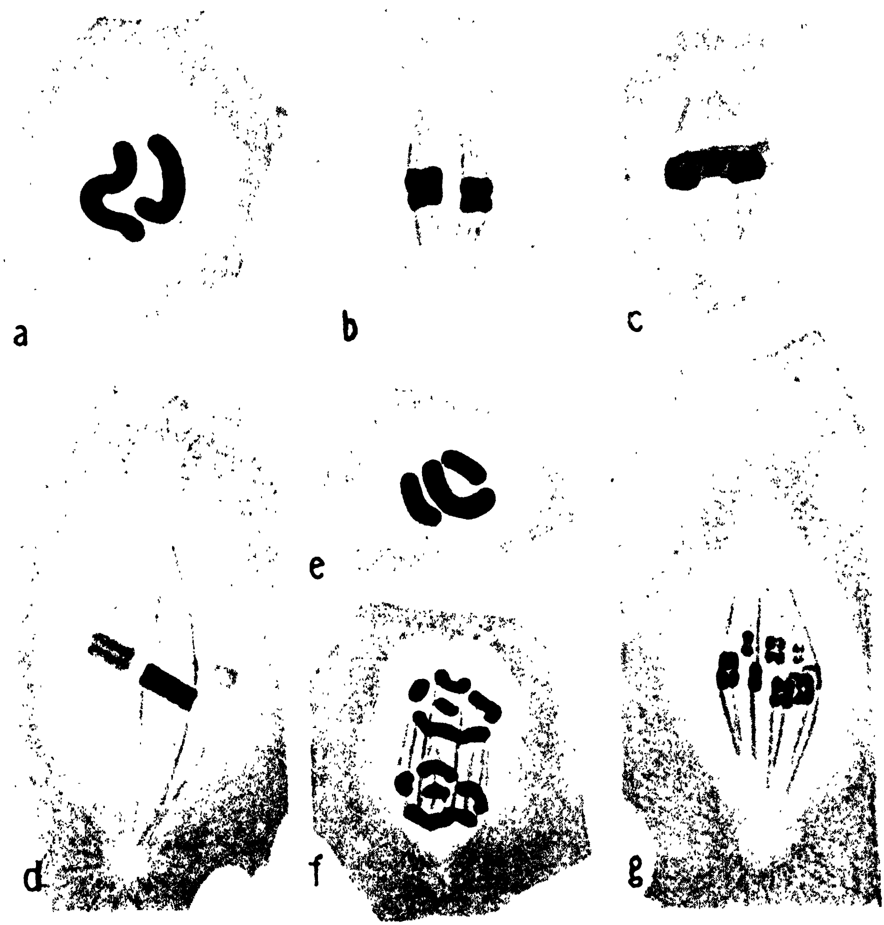


FIG. 2

Mitotic potency of chromosome fragments; normal and irradiated embryonic cells of Steatococcus. *a, b, c*, normal metaphase from untreated haploid male embryo (n = 2); *d, e*, single break in one chromosome of haploid embryo nucleus — normal orientation and chromosomal fiber formation by fragment; *f*, parallel anaphasic disjunction of fragments; *g*, lateral aspect of metaphase in cell with both chromosome and chromatid fragments. From Hughes-Schrader and Ris (1941).

in many other forms in which no complete analysis has yet been made suggests a diffuse condition of the kinetochore. Even when a typical localized kinetochore is present other regions of the chromosome may

evince kinetic potency as in meiotic divisions of maize plants with an abnormal 10th chromosome (Rhoades and Vilkomerson, 1942) and certain strains of rye (Prakken and Müntzing, 1942; Östergren and Prakken, 1946).

On the tenuous basis of the bending of the chromosome ends toward the pole in late anaphase, Piza (1943) has maintained that the coccid chromosome has two terminal kinetochores. In order to account for the mitotic potency of fragments and the structure of the half spindle, however, he is forced to postulate a diffusion of kinetic influence from the ends along the whole chromosome, which is tantamount to a recognition of the diffuse nature of the kinetochore.

The existence of the diffuse type of kinetochore has also been questioned on the ground that were no localized kinetochore present, the chromosome should "obey quite different laws from those which govern the evolutionary transformations of other chromosomes. So far there is no indication that this is the case" (White, 1945, p. 22). It has been shown that the chromosome with a diffuse kinetochore does indeed obey different laws in its behavior — in respect to orientation, chromosomal fiber production, and anaphase disjunction, in short, the very features that one would expect to be influenced by the kinetochore condition. That different laws also govern its evolutionary performances is already evident. Thus, simple fragmentation of the chromosome with a diffuse kinetochore must be reckoned as a possible mechanism for evolutionary increase in chromosome number, as witness Troedsson's (1944) work on compound sex chromosomes in Heteroptera (further discussion in Schrader, 1947). It will be further evident in the ensuing review of the meiotic mechanism in different groups of coccids that the evolution of linear aggregate types of spindles, chromatid autonomy, asynapsis, and the modification of bivalent structure and orientation have all been either dependent on or facilitated by the diffuse kinetochore.

III. Primitive Pattern of Meiosis in Coccids

A. *Male Meiosis*

Fortunately there persists in Puto sp. (family Eriococcidae) a simple and regular meiosis in both sexes, which from its generalized character may well prove archetypal for coccids (Hughes-Schrader, 1944). The sex chromosome mechanism is of the XX-XO type; the chromosome complement of the female is 2n = 14, of the male 2n = 13.

Although pre-diakinetic stages are not analyzable in the male Puto a normal synapsis may be assumed from the regularity of bivalent formation. At diakinesis the bivalents have either already assumed the rod form with a single terminal association between the homologous chromo-

somes, or the form of the open cross (fig. 3, *a*, and 4, *a*). Whatever chiasmata may have been present are completely resolved by metaphase. But the coccid bivalent differs from the usual rod bivalent in two respects, both brought about by the diffuse kinetochore. First, sister chromatids and half chromatids are free to separate throughout their length; and second, there is no localized "repulsion effect" on bivalent shape such as is associated with the ordinary kinetochore. At metaphase the coccid bivalent thus comprises two quartets of parallel rods in tandem associations. This basic structure, diagrammatically clear in certain primitive llaveiines (fig. 17, 2) is obscured in Puto by extreme condensation and close association of the elements of the bivalent which at metaphase has the appearance of two pairs of almost spherical moieties in close apposition (fig. 4, *b*, *c*).

The essential and distinguishing features of the meiotic divisions may be summarized as follows: (1) The first division is equational for all non-crossover regions; (2) the chromatids disjoin and realign in a secondary pairing; and (3) the second division is reductional for non-crossover regions.

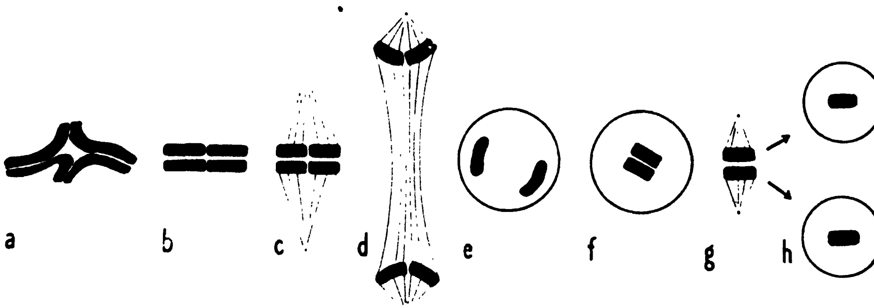


FIG. 3

Diagram coccid-aphid type of meiosis. *a*, *b*, a typical bivalent in diakinesis; homologous chromosomes assume end to end association; *c*, metaphase I, with independent orientation of each chromosome of the bivalent; *d*, anaphase I, with equational disjunction of non-crossover regions; *e*, disjunction of chromatids in interkinesis; *f*, secondary, parallel, pairing of disjoined chromatids; *g*, metaphase II; *h*, second division, reductional for non-crossover regions.

This is brought about in the following way. At first metaphase the bivalents orient with the long axis of each chromosome on the equator of the spindle. The split visible in a polar view (fig. 4, *b*) is thus the primary split, here the point of contact between the ends of the homologous chromosomes. The constriction visible in lateral aspect (fig. 4, *c*) is the secondary split between sister chromatids. Thus, instead of a co-orientation above and below the equator of the two homologous kinetochores, such as occurs in most organisms, each chromosome of the coccid bivalent is independently

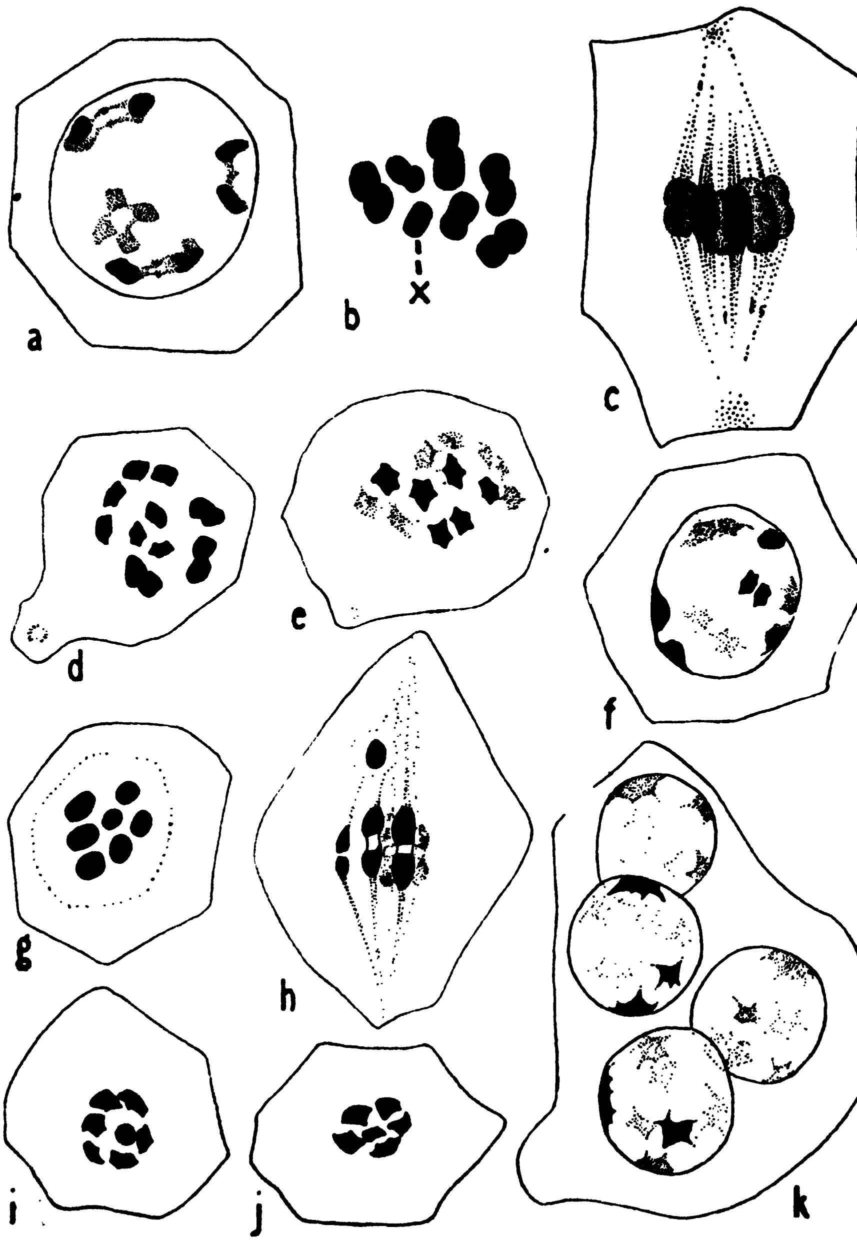


FIG. 4

Meiosis in male Puto. *a*, diakinesis; *b*, *c*, metaphase I; *d*, *e*, disjunction of chromatids in interkinesis; *f*, secondary pairing of homologous chromatids; *g*, *h*, *i*, second division; *k*, quadrinucleate spermatid. From Hughes-Schrader (1944).

oriented. The first division is accordingly equational for all non-crossover regions.

Interkinesis witnesses two unique and significant maneuvers. First the two chromatids of each dyad disjoin completely (fig. 4, *d*, *e*). Second, after slight uncoiling, homologous chromatids again approach each other in a secondary pairing (fig. 4, *f*). That this pairing is a side by side or parallel alignment is conclusively demonstrable in Nautococcus (page 169); in Puto most of the chromatids are too short for positive identification of the axis. After pairing, orientation for the second division, again conditioned by the diffuse kinetochore, brings the plane of secondary pairing into the equator and segregation of non-crossover regions results (fig. 4, *f–i*). These relations are diagrammatically summarized in Figure 3.

The male Puto thus presents a fairly primitive pattern of the aphid-coccid type of meiosis first analyzed by Ris (1942), and provides an intelligible basis for an understanding of the evolution of the meiotic mechanism in the group as a whole. This type of meiosis must have originated after the separation of Sternorhynca and Auchenorhynca in the homopteran stem since it is present in the former only and not in the latter. Neither is it found in Heteroptera. Its development is thus not a necessary sequel of the diffuse kinetochore, although obviously the independent orientation — rather than the usual co-orientation — of meiosis would be facilitated by it. In the Odonata, the only other group in which the aphid-coccid sequence of division and segregation in meiosis has been encountered, Oksala (1943) believes the kinetochore to be of the localized type. The parallel anaphase disjunction and peculiar realignment of chromatids at interkinesis would however be more readily intelligible on the assumption of a diffuse kinetochore; experimental verification is desirable.

The only specialized feature of the Puto meiosis lies in the formation of quadrinucleate spermatids by the cytoplasmic fusion of the four derivatives of each primary spermatocyte (fig. 4, *k*). This process occurs in all coccids thus far studied. Its regularity depends on the persistence, in the radially arranged cells of each cyst, of a centrally directed cytoplasmic stalk in each spermatocyte. This stalk is never completely severed in the meiotic divisions and provides a limiting factor in the later cytoplasmic fusion as is demonstrable in Protortonia and Llaveia (Schrader, 1931; Hughes-Schrader, 1931).

B. *Female Meiosis*

In contrast to its diverse modifications in the male line a primitive meiosis has been retained by the female in many coccids. Thus the formation of typical bivalents and the production of two polar bodies with reduction of the chromosomes to the haploid number have been verified in

some 20 species among 3 families. Technical difficulties preclude detailed analysis in many of these (including Puto), but so uniform is the course of meiosis that a description based on the technically most favorable (Schrader,



FIG. 5

Öogenesis in *Pseudococcus citri*. *a*, *b*, diakinesis; *c*, metaphase I; *d*, early anaphase I; *e*, late anaphase I; *f*, telophase I, with progressive disjunction of chromatids; *g*, metaphase II, secondary pairing complete; first polar body in division above. After Schrader (1923a).

1923a; Thomsen, 1927; Suomalainen, 1940) may safely be assumed to apply to all species retaining normal meiosis.

As in the male, prediakinetic stages have not been analyzed but normal pairing is indicated by the constancy of the full haploid number of bivalents at diakinesis. At the latter stage the bivalents have the form of the open

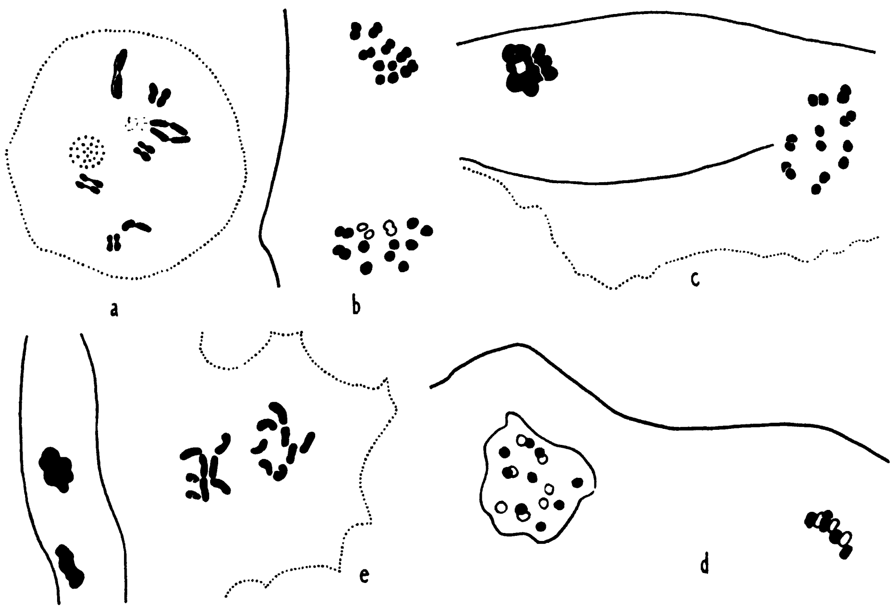


FIG. 6

Oögenesis. *a*, diakinesis in *Gossyparia spuria* — end to end association of homologues in each bivalent; *b–e*, *Lecanium hemisphaericum*; *b*, disjunction of chromatids in anaphase I; *c*, early metaphase II, secondary pairing in progress; *d*, late metaphase II, secondary pairing complete; polar body at left; *e*, anaphase II showing parallel disjunction of parallelly paired chromatids. *a*, after Schrader (1929a); *b–e*, after Suomalainen (1940).

cross (fig. 5, *a*). With progressive coiling the short arms of the cross are eliminated as the homologous chromosomes assume an end to end association (fig. 6, *a*; 8, *a*). Sister chromatids separate completely, but half chromatids have not been reported to do so in female meiosis. In most species coiling continues until long and short axis of the bivalent are no longer distinguishable. But fortunately in *Lecanium hesperidum* (fig. 8, *b*) condensation is less extreme and it can be seen that the long axis of the bivalent lies in the equator. The first division is accordingly here, as in the male, equational for non-crossover regions. That this holds true for the other species also appears certain from their close correspondence in bivalent structure and behavior.

Again paralleling male meiosis, late anaphase and interkinesis invariably witness disjunction of the chromatids of each dyad (figs. 5, *d-f*; 6, *b*; 8, *c*). This is followed, with no intervening resting stage, by a secondary pairing of the often widely separated chromatids (fig. 5, *g*; 6, *d*). The re-formed dyads then orient at once for the second division. The significance of these curious maneuvers of disjunction and reassociation, which were conclusively demonstrated by both Schrader (1923a) and Thomsen (1927), could not be appreciated at the time their studies were made. It is now clear that they constitute an adaptive mechanism ensuring that realignment of chromatids essential to consummation of reduction in this type of meiosis. The parallel nature of the secondary pairing is fortunately demonstrable in *Lecanium hemisphaericum* (Suomalainen, 1940). Although here as in most species the compactness of metaphase chromatids precludes a recognition of their axes, early in second anaphase the chromosomes reassume the elongate form of somatic mitosis. In the parallel disjunction of second anaphase (fig. 6, *e*) it is clear that the long axis of each chromatid lies parallel to the equator, and that the preceding secondary pairing was of necessity parallel.

To summarize: The primitive pattern of female meiosis is essentially the same as in the male. Bivalents are of the rod type with no kinetochoric connection between sister chromatids. Sequence of division and segregation is identical with that of the male. Each chromosome of a bivalent orients independently at first metaphase; co-orientation of homologous chromatids at second metaphase follows a secondary, and parallel, pairing in interkinesis.

IV. Modifications of Meiosis in Female

A. *Parthenogenesis*

Although more conservative in evolution than its counterpart in the male line, meiosis in female coccids presents a series of modifications which would be considered impressive in a group of only average plasticity. Most of these modifications involve the removal of the block to development in unfertilized eggs and have resulted in parthenogenesis of several distinct types. To judge from collection and breeding data such changes have occurred frequently and independently in many sections of the group. In evaluating these data it should be remembered, as Winkler (1920), Schrader (1923a), and Thomsen (1927) have emphasized, that an unbalanced sex ratio or even total absence of males in collections may stem largely from the fragility, inconspicuousness, short adult life, and different habit of male as compared with female coccids. Furthermore, James' (1937, 1938) experiments on species of Pseudococcus have demonstrated a direct effect

of environmental factors on sex ratio which is correlated neither with differential mortality nor with parthenogenesis. And finally, since functional hermaphrodites indistinguishable externally from normal females are known within the group, even the production of young from unmated mothers does not constitute proof of parthenogenesis. But even within the limits of the cases thus far checked cytologically, parthenogenesis has been demonstrated in 13 species representing 3 families, and its occurrence is highly probable in still others. This is not surprising since the adaptive value of parthenogenesis should be unusually high in coccids, both on account of the short adult life and fragility of the male, noted above, and because of the frequent asynchrony in sexual maturity in male and female. The latter stems from the loss — never completely compensated in many species — of the final instar in female metamorphosis.

Three major types of parthenogenesis are now known to occur: (1) obligate diploid thelyotoky, (2) facultative diploid thelyotoky, and (3) facultative haploid arrhenotoky. In addition several species have become differentiated into unisexual and bisexual races characterized respectively by the first and second type of reproduction.

1. Obligate Diploid Thelyotoky

Obligate diploid parthenogenesis giving rise to females only has been demonstrated cytologically and by breeding experiments by Thomsen (1927, 1929) in one race of each of two species of Lecanium (family Lecaniidae), by Schrader (1929b) in *Aspidiotus hederae* (family Diaspididae), and by Hovasse (1930) in *Marchalina hellenica* (family Margarodidae). The cytological mechanism appears to be the same in all. The following account is based on *Lecanium hesperidum* and *Aspidiotus hederae* on which the cytological data are complete.

No bivalents are formed in meiotic prophase. At the stage corresponding to diakinesis in sexual eggs, the egg nucleus contains univalents only, and these in the full diploid number of the species (fig. 7, *a*). A single meiotic division, equational in type, gives rise to a single polar body and the egg pronucleus. The latter without any further meiotic development sinks into the egg and becomes the first cleavage nucleus (fig. 7, *b–e*). The diploid garniture and female genome of the mother is thus preserved intact in the offspring through the suppression of bivalent formation and of the second meiotic division. That the one maturative division of the egg nucleus is of meiotic origin is obvious since a typical polar body is formed; that it retains some meiotic features is shown by the fact that the chromosomes, although unpaired, undergo the extreme coiling and condensation of meiotic as contrasted with somatic prophase (compare meiotic prophase in haploid males, page 158). It is of interest also that the same

genetic setup which suppresses synapsis here likewise inhibits secondary pairing. In contrast, in interkinetic nuclei of sexual eggs, which are also diploid (through disjunction of previously synapsed chromatids) a second-

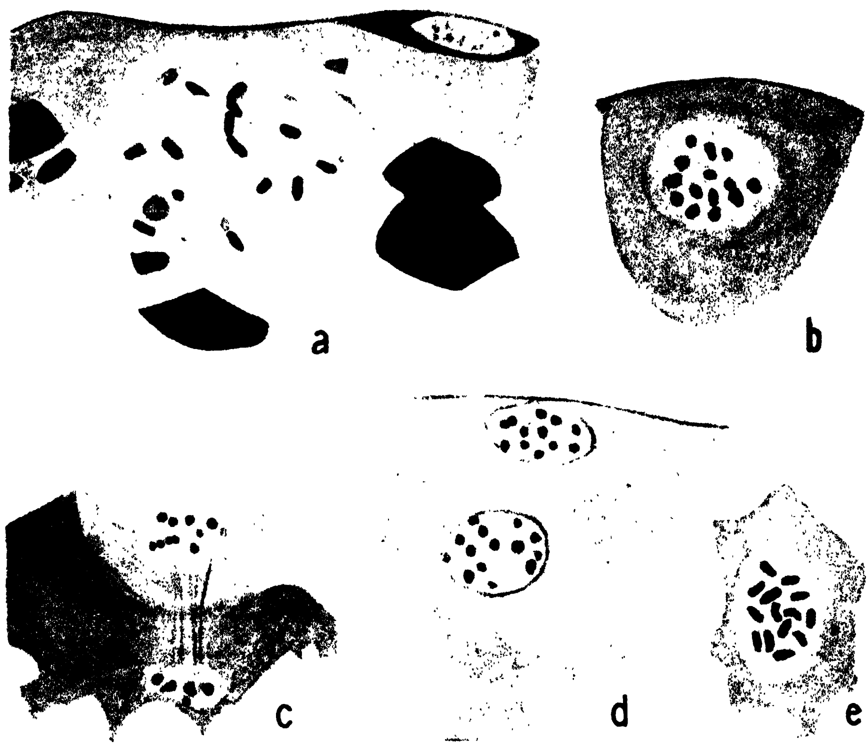


FIG. 7

Obligate diploid thelyotoky in *Lecanium hesperidum* (2n = 14). *a*, meiotic prophase; *b*, metaphase, each with diploid number of univalents; *c*, anaphase; *d*, telophase; *e*, first cleavage nucleus (= inner telophase nucleus). After Thomsen (1927).

ary pairing re-establishes pseudo-reduction and a second meiotic division ensues. In the meiosis of the obligatorily parthenogenetic egg, both secondary pairing and the ensuing division are completely suppressed.

2. Facultative Diploid Thelyotoky

Of even greater interest for cytology in general, because of its relative rarity and the clarity of the demonstration here possible, is a second type of diploid parthenogenesis discovered by Thomsen (1927, 1929) in several species of Lecanium. Here a normal meiosis occurs in all eggs, with a reduction of the chromosomes to the haploid set. Diploidy is then restored by the fusion of the second polar body with the egg pronucleus. The

essential points in the cytological evidence are shown in Figure 8. Bivalent formation, and both first and second meiotic divisions are seen to be of the normal coccid type. The critical stage is shown in Figure 8, *e*. Here

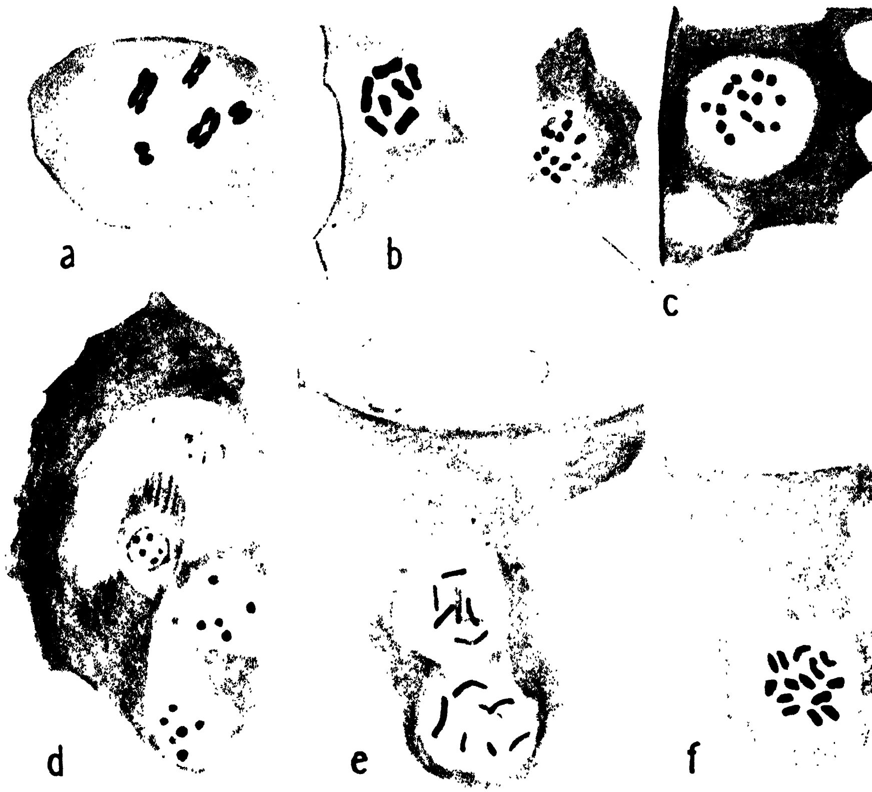


Fig. 8

Facultative diploid thelyotoky in *Lecanium hesperidum* (2n = 14). *a*, section through diakinetic nucleus showing 5 of the 7 bivalents; *b*, metaphase I — 7 bivalents; *c*, anaphase I, with disjunction of chromatids in progress (from two adjacent sections); *d*, anaphase II — with first polar body in telophase above; *e*, approach of second polar body and female pro-nucleus; *f*, first cleavage nucleus from fusion of second polar body and female pro-nucleus. After Thomsen (1927).

the egg pronucleus moves towards the center of the egg, closely followed by the second polar body. These nuclei then coalesce and the resultant fusion nucleus becomes the first cleavage nucleus of the developing embryo. Not only is Thomsen's identification of the nuclei involved critically supported and his seriation of stages rigorously checked, but in addition the whole process was followed in eggs of females grown in complete isolation from

males. Any possibility of confusing the second polar body with a sperm pronucleus is thus excluded.

The crucial step in the evolution of this type of parthenogenesis would seem to lie in the activation of the second polar body, — which now functions like a sperm nucleus in the initiation of cleavage. That this is more than a simile is shown by the fact that the potential sexuality of the egg is not lost. If sperm are present a normal fertilization is consummated. (This statement should be qualified. Actual fusion of male and female pronuclei has not been seen in Lecanium; — but eggs containing sperm heads in close proximity to the egg pronucleus were recovered and showed no activity of the second polar body. This, together with the production of males in wild colonies and their total absence in offspring of isolated females, warrants the assumption.) We must therefore further infer an inhibiting effect of the sperm on the activity of the second polar body.

Both the obligate and the facultative types of parthenogenesis result in diploid offspring. The first maintains diploidy by an almost complete suppression of meiosis with consequent loss of the sexual potency of the eggs; the second restores diploidy by a fusion of polar body and female pronucleus, retaining sexual potency. In both the parthenogenetically produced offspring are of course female. The two types should however behave differently genetically, since the facultative type permits recombinations of genes in the offspring of a heterozygous mother, while the obligate type precisely reproduces the maternal setup, if the possibility of newly arisen changes be disregarded. (Further discussion in Suomalainen, 1940; White, 1945.)

3. Parthenogenesis and the Differentiation of Races

The differentiation of races within a species on the basis of reproductive processes and in relation to distribution and ecology constitutes a promising field of coccid biology the exploration of which is barely begun. The following account is confined to cases for which some cytological data are available. In *Lecanium hesperidum* and *Lecanium hemisphaericum* males are rare throughout the range, but the sex ratio varies in different regions and in different colonies in the same region. Thomsen (1927) has shown that in each species there exist two races anatomically indistinguishable but radically different in reproduction and cytology. One race comprises females only and reproduces exclusively by the obligate diploid parthenogenesis described in section 1 above. The other is bisexual, producing both females and, in smaller number, males. But when isolated from males, females of the bisexual race reproduce parthenogenetically, and they too

give rise to females only, but by the very different cytological mechanism described in 2. Effective isolation of the two races is assured by the suppression of meiosis in the obligatorily parthenogenetic race.

Absence of males in a colony does not in itself however indicate the existence of an obligatorily parthenogenetic race. Should a new territory be invaded by virgin females of the bisexual race, the resulting colonies would be exclusively female and indistinguishable except cytologically from the obligate parthenogenetic race. Precisely this situation has apparently developed in an allied Lecanium species, *L. coryli*. In Denmark, Thomsen (1929) found females only, although both sexes are reported throughout most of the range of the species in central and southern Europe. It would be natural to assume that here, as in *L. hesperidum* and *L. hemisphaericum*, two races are involved — an obligate parthenogenetic race in Denmark and a bisexual facultatively parthenogenetic race in central Europe. But a cytological check of females from Denmark, Germany and Italy, shows that all belong to the latter type; Denmark must have been colonized by virgin females from central Europe.

Males are rare and sex ratios variable in *Lecanium corni* also. Here again Thomsen (1929) finds only the bisexual type of female — alike in the unisexual colonies of Denmark and in a bisexual colony from Germany. Thiem (1933) in an extensive study of sex ratios in wild populations of *L. corni* (= *Eulecanium corni*) reports that the number of males in a colony varies from 0 to 25 per cent and is correlated with altitude and temperature, and variably affected by weather during the eclosion of males. His conclusion that races are not here involved, but that sex ratio is directly conditioned by environmental factors, is not warranted since no cytological study of the different populations was made. Thiem's further conclusion that *L. hesperidum* also is racially undifferentiated and reproduces unisexually or bisexually according to environment is untenable and rests on his misconception of Thomsen's evidence (critique in Suomalainen, 1940).

Schrader's (1929b) demonstration in *Aspidiotus hederae* of obligate diploid parthenogenesis resulting in exclusively female colonies was made on American greenhouse stock. That a bisexual race also exists is indicated by the occurrence of males in Russian stock studied by Gabritchevsky (1923), who also reports a difference in sex ratio correlated with host plant species. No cytological data are available on this latter race.

In *Marchalina hellenica* reproduction is obligatorily parthenogenetic in the only stock thus far studied cytologically (Hovasse, 1930); but here no males have ever been encountered in nature, nor in the extensive life history studies of Vayssière (1926). It is thus probable that in this species the ancestral bisexual race no longer exists.

4. Facultative Haploid Arrhenotoky

A type of parthenogenesis differing radically from the diploid thelyotokous mechanisms just discussed is encountered in the margarodid tribe, Iceryini. Here all eggs undergo a normal meiosis but cleavage is initiated and maintained without restoration of diploidy. Parthenogenetically produced offspring are thus haploid, and are invariably male, while fertilized eggs give rise to females only. Diploid males, such as occasionally arise from fertilized eggs in certain Hymenoptera, have never been encountered in these coccids (discussion relative to sex determination, page 193). The evolutionary origin of this facultative haploid arrhenotoky clearly involves a more complicated series of correlated changes than does thelyotoky. In the latter we need assume only (1) removal of the block to development of the unfertilized egg and (2) evolution of a mechanism for maintenance or restoration of diploidy. For arrhenotoky on the other hand, the prerequisite correlated modifications as summarized by Schrader and Hughes-Schrader (1931) include: (1) initiation of development without fertilization; (2) acquisition of viability in the haploid state, (and the data of artificial parthenogenesis indicate that the normal block to such viability is strong in most metazoans); (3) establishment of the haplo-diploid sex determining mechanism; and (4) regulation of meiosis in the haploid male for the production of normal gametes. It is therefore not surprising that these conditions have been but infrequently fulfilled; haploid arrhenotoky is rare as compared with thelyotoky.

Among coccids a single evolutionary origin of arrhenotoky is indicated since it is known solely in one closely related group of genera within the tribe Iceryini of the family Margarodidae. It has been demonstrated in the following species: *Steatococcus tuberculatus* (Hughes-Schrader and Ris, 1941), *Echinicerya anomala*, *Crypticerya rosae*, *Icerya montserratensis*, *I. littoralis* (Hughes-Schrader, 1930a and b), *I. similis* (Hughes-Schrader unpub.), and in the hermaphroditic species *I. purchasi* (Hughes-Schrader, 1926, 1927; Schrader and Hughes-Schrader, 1926). This group of iceryines constitutes almost ideal cytological material which is especially fortunate in view of the technical difficulties impeding analysis in most other organisms with this type of reproduction. Complete uniformity in chromosome size, number, and with the notable exception of *Icerya purchasi* to be discussed later, in chromosome behavior characterize the species listed. The following summary of their cytology based on *Icerya littoralis* (Hughes-Schrader, 1930a) applies to all, with certain exceptions in the case of *I. purchasi*.

The diploid chromosome number of the female is 4, comprising two pairs distinguishable in length. Males carry the haploid number of but 2 chromosomes identifiable by length as one member of each pair of the

female set (fig. 9). The haploidy of the male has been directly confirmed for many somatic tissues as well as spermatogonia in all instars in which sex is distinguishable. In stages prior to sexual differentiation male haploidy is also, if indirectly, established since these stages in the offspring of virgin females are always haploid and the offspring of virgin females are

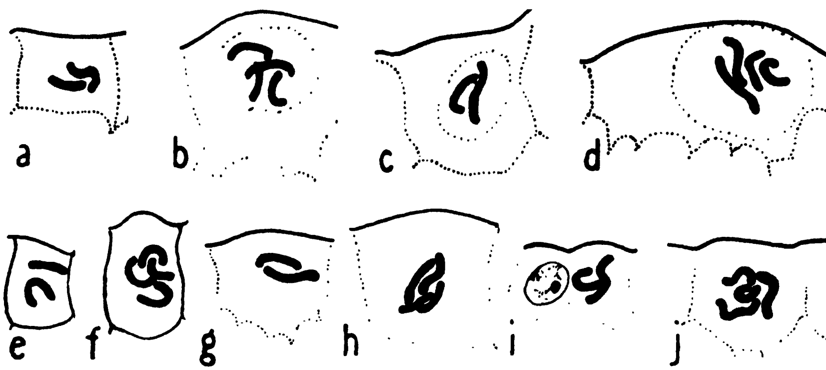


FIG. 9

Haplo-diploidy of Iceryini. *a*, male, *b*, female somatic chromosome complement of *Icerya littoralis*; *c*, male, *d*, female, of *I. purchasi*; *e*, male, *f*, female, of *I. montserratensis*; *g*, male, *h*, female, of *Echinicerya anomala*; *i*, male, *j*, female, of *Crypticerya rosae*. From Hughes-Schrader (1930a).

always male. This is not to say that occasional somatic polyploidy does not occur; indeed it is frequent in both sexes especially in gland cells, intestinal epithelium, and mycetocytes. No data are available on the degree of polyploidy in corresponding tissues of the haploid and diploid sex. Although no careful comparative study has been made, polyploidy appears to be more frequent in males than in females. This is certainly true in the male *Echinicerya anomala* where nests of polyploid cells are frequent in all somatic tissue. But even here no general regulation of surface-volume relations of cells through restoration of diploidy has taken place; even adult males remain predominately haploid in soma, and invariably so in germ tract.

That the haploid male develops parthenogenetically from a reduced, unfertilized egg has been demonstrated in combined breeding and cytological studies. Meiosis was followed in parallel series of eggs from virgin and from fertilized females. In both series all eggs undergo normal meiosis; two bivalents are formed, and two polar bodies are given off (compare fig. 11 from *Icerya purchasi* — female meiosis is identical in all iceryines studied). In all eggs of a virgin female cleavage is then initiated by the haploid female pronucleus; the resulting embryos are haploid, the resulting

offspring male. In eggs of a fertilized female, sperm are found to enter the egg at the time of first meiotic metaphase. On the conclusion of meiosis male and female pronuclei fuse and the resultant diploid nucleus immediately initiates cleavage. The diploid offspring are invariably female. A variable number of eggs escapes fertilization in mated females, and these undergo the same haploid parthenogenetic development as in virgin females, and give rise to males.

What determines this variable production of males by a fertilized female is not known. Mechanical obstruction or belated opening of the ovarioles with consequent delay of sperm entry until after the initiation of parthenogenetic development may be a factor in some cases, although in *Echinicerya anomala* sperms are able to penetrate into the ovariole before it opens. Insufficiency of sperm appears improbable (compare *Icerya purchasi*, page 153). Since the eggs develop over a long period it is conceivable that time of mating may modify sex ratio (compare effect of aging of female on sex ratio in Pseudococcus, page 192). Certainly there is no evidence of control of fertilization by the female as in the bee; a spermatheca is present but is apparently of little or no function since the majority of the sperm bundles are deposited directly into the oviducts or central cavity of the ovary. The regulation of meiosis in the haploid male for the production of normal gametes is completely stabilized; the cytological evidence is reviewed in connection with other modifications of male meiosis (page 158). The haploid male-diploid female type of sex determination here associated with arrhenotoky is discussed on page 193. In so far as modifications of the female mechanism are concerned, the only cytologically demonstrable change from the primitive bisexual condition lies in the removal of the block to post meiotic mitosis in the haploid egg pronucleus.

B. *Hermaphroditism*

Most dramatic of the many surprises of coccid cytology was the discovery of functional hermaphroditism in the cottony-cushion scale, *Icerya purchasi* (Hughes-Schrader, 1925, 1926, 1927). Earlier studies had shown extreme rarity of males; Pierantoni (1910, 1914) had reported parthenogenesis and Kuwana (1922) had also succeeded in rearing several generations of females in the complete absence of males. Thelyotoky certainly seemed indicated. The first cytological studies on this species are those of Pierantoni (1910, 1911, 1912, 1914) who correctly established the chromosome number of the female. He further described an obligate diploid parthenogenesis involving two equational divisions in female meiosis. Pierantoni (1911, 1914) also noted the occurrence of an hermaphrodite phase which he believed to be a transient stage in the testes of young male nymphs. Reinvestigation by Hughes-Schrader (1925, 1926,

1927), using both breeding and cytological techniques showed the supposed females to be self-fertilizing hermaphrodites. The males, which are never hermaphroditic, are haploid and develop parthenogenetically from unfertilized reduced eggs of the hermaphrodite. They conform in all respects to the iceryine type (page 157). The hermaphrodites, comprising the bulk of the population, are diploid and arise from fertilized eggs only. They are basically female as is evident in their close correspondence in body form, habit, and mating instinct with the true females of allied species.

The chromosomes of *Icerya purchasi* are indistinguishable from those of the other iceryines already reviewed. The diploid number of the hermaphrodite is 4, with the same size difference between the two pairs; the haploid nucleus of the male carries one chromosome of each pair (fig. 9, *c–d*). The hermaphrodite gonad, sexually undifferentiated during the embryonic period, then passes through a predominately male phase, and finally into a female phase. The differentiation is spatial, as well as temporal; the male tissue forms in general the central part of the gonad, with the female tissue enveloping it.

Male differentiation is initiated early in the first instar by the appearance of a few haploid nuclei in the developing gonad. Unfortunately the mechanism of this premeiotic reduction remains unknown. No pairing, no elimination, of chromosomes has been seen. A certain amount of degeneration of whole nuclei occurs but this is definitely not correlated with the reduction, occurring alike in male and hermaphrodite. Of the actuality of the reduction there can, however, be no doubt; the chromosomes of the haploid nuclei are identifiable by size and shape as one member of each pair of the diploid set. Rapid proliferation of these haploid cells gives rise to the more or less continuous central core of spermatogenous tissue (fig. 10). The spermatocytes undergo a single, equational, meiotic division and two normal sperms are formed from each. The male meiosis is thus identical with that of the true males (compare page 157). As the sperms mature they aggregate into compact bundles which occupy far less space than the spermatocytes they replace (fig. 10, *b*). The gonad is thus hollowed out by the very process of spermiogenesis. It is transformed from a solid structure, not unlike — except for its mantle of diploid oogenetic cells — the testis of the true male into a hollow organ from whose walls the ovarioles develop. It is then essentially similar, except for its cargo of sperm bundles, to the ovary of the typical iceryine female.

Diploid cells trapped in haploid areas usually fail to differentiate into meiocytes of either sex, although occasionally early-degenerating oocytes and nurse cells may develop from them. In only two hermaphrodites in several hundred studied was spermatogenesis in diploid cells observed. This aberrant behavior was reported as normal in Hughes-Schrader's first

account (1925); the error is corrected in later reports (Hughes-Schrader, 1926, 1927; Schrader and Hughes-Schrader, 1926). Unfortunately the diploid material is too limited for complete analysis. It comprises (1)

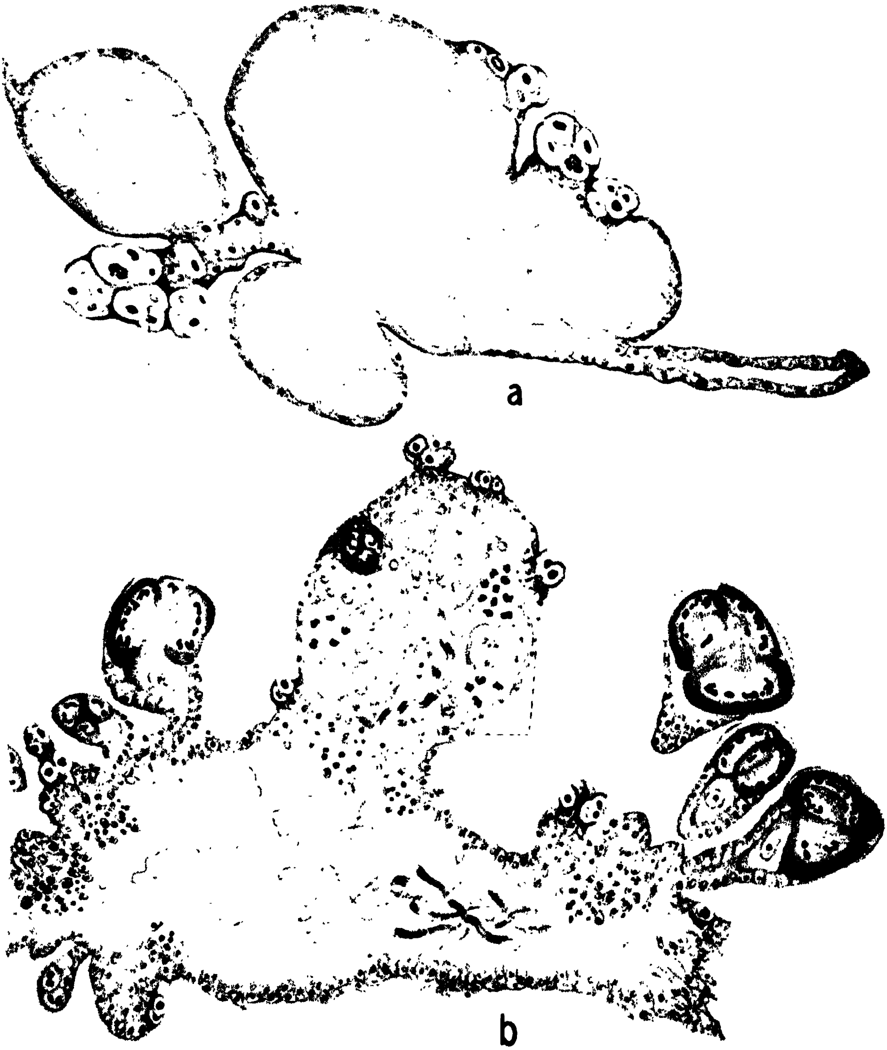


FIG. 10

Hermaphroditic gonad of *Icerya purchasi*. *a*, part of longitudinal section through second instar gonad; duct at right; solid masses are spermatogenous (haploid); peripheral nodules are oogonia (diploid) in early stages of ovariole formation; *b*, same, from third instar gonad; central cavity with sperm bundles; mid top, a mass of spermatogenous cysts (haploid) with meiosis in progress; ovarioles (diploid) peripherally — the older ones differentiated into oocyte and nursecells. From Hughes-Schrader (1925).

some diploid prophases, (2) binucleate cells interpreted as telophases of the first meiotic division, (3) cells with two anaphasic spindles and two chromosomes at each pole, and (4) quadrinucleate spermatids such as would result from (3). It was concluded that two meiotic divisions including chromosome reduction (the latter process not represented in the actual material) would occur were the diploid number of chromosomes present in the spermatogonia (Hughes-Schrader, 1927, p. 520). Were this interpretation correct, we would be justified in assuming with Whiting (1945) and White (1945), that the haploid type of meiosis is not genotypically determined in the iceryines but is simply a mechanical consequence of the haploid condition. It is the reviewer's present opinion that this interpretation is not justified, and for the following reasons. The number of nuclei per cyst is extremely constant in iceryine spermatogenesis. The diploid cysts number 8 "primary spermatocytes" and 32 spermatid nuclei; haploid cysts invariably contain 16 spermatocyte and 32 spermatid nuclei. The diploid cysts therefore probably arose by fusion or incomplete cytoplasmic division of already reduced, haploid cells. The mitosis that increased the nuclei per cyst from 8 to 16 was then in reality the last spermatogonial mitosis, and gave rise to the binucleate cells noted in (2) above. These nuclei then underwent the typical haploid division, seen in the twin anaphases of (3) above. This interpretation fits the available stages without necessitating the assumption of an unobserved pseudoreduction; it is further supported by the fact that similar behavior has been experimentally induced in Llaveiella spermatocytes (Hughes-Schrader-unpublished data). Here the two chromosome sets in cold-induced tetraploid (syndiploid) spermatocytes occasionally form separate spindles and the resulting anaphasic configurations precisely parallel those of the "diploid" Icerya spermatocytes, which are more probably interpretable as synhaploids. The weight of evidence favors the conclusion that (1) male differentiation in the hermaphrodite is dependent upon a prior establishment of haploidy, and (2) the haploid type of meiosis in the iceryines is genotypically determined.

Female differentiation in the hermaphroditic gonad is initiated with the development of definitive oogonia during the latter half of the male phase. They are distinguishable cytologically not only by their large size but also by a curious "continuous spireme" formed in prophase by the end to end alignment of the chromosomes. (Compare the similar "terminal attraction" between chromosomes in spermatid and sperm of iceryines, page 159.) From each of these oogonia develop the nurse cells and oocyte of the typical coccid ovariole. Meiosis is normal, of the characteristic coccid type; two polar bodies are formed, and the chromosomes reduced to the haploid set. The hermaphroditically produced sperm enter the ovariole

in large numbers, as the growth of the egg brings the ovariole into open communication with the central cavity of the gonad in which the sperm bundles lie. The eggs are fertilized while still in the ovarioles; deposition of eggs and formation of egg sac proceed as in true females of related species.

Conclusive proof of self fertilization was obtained in a series of hermaphrodites isolated from time of hatching (Hughes-Schrader, 1925).

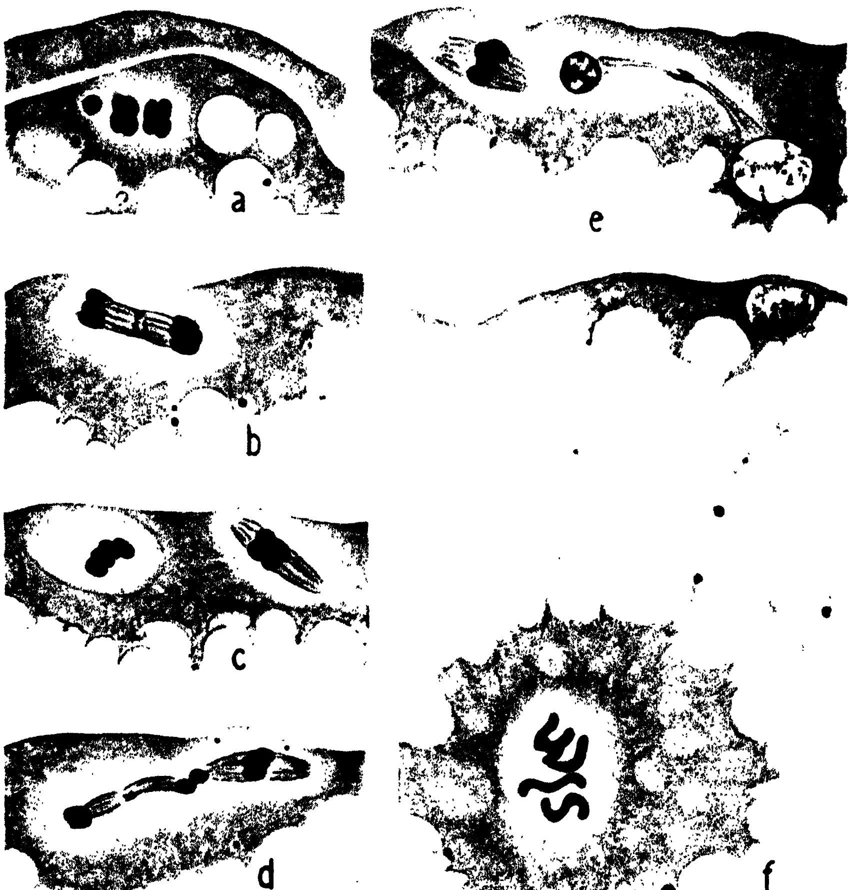


FIG. 11

Öogenesis and self-fertilization in the hermaphrodite *Icerya purchasi*. All figures from eggs of hermaphrodites reared in isolation from males. *a*, metaphase I; normal bivalent formation; *b*, anaphase I; *c*, metaphase II with first polar body in division; *d*, anaphase II, reduction of chromosomes to haploid number; first polar body upper right; *e*, telophase II, female pronucleus at right; *f*, self-fertilization; diploid fusion nucleus from union of hermaphroditically produced sperm nucleus and female pronucleus; second polar body at periphery, first polar body in adjacent section. From Hughes-Schrader (1925).

Closely seriated stages in the meiosis of the eggs, development of the sperm, penetration of egg by sperm, and fusion of male and female pronuclei were observed in the gonads of these "virgin" hermaphrodites, providing a cytological demonstration of self fertilization (fig. 11). Precisely similar is the course of events in hermaphrodites which have mated with males. This is to be expected, since protandry assures the hermaphrodite's own sperm preferential position, priority, and a vast majority in numbers. Fertilization by the sperm of a male must be a rare occurrence. Confirmation is afforded by interspecific crosses of the hermaphrodite of *I. purchasi* with the male of *I. littoralis* (unpublished data, Schrader and Hughes-Schrader), which yielded only *I. purchasi* hermaphrodites.

All fertilized eggs give rise to hermaphrodites. The rare males, as noted above, develop parthenogenetically from eggs which escape fertilization. As would be expected from the cytological data, breeding experiments (Hughes-Schrader, 1926, 1927) show that the number of males is not affected by mating of hermaphrodite and male. The persistence of males in *I. purchasi*, albeit in small and variable numbers, and the facultative arrhenotoky giving rise to them, are simply a part of the iceryine heritage and no longer necessary to the perpetuation of the species. It would therefore not be surprising to find an exclusively hermaphroditic race differentiating through the loss of parthenogenetic potency. The data at hand are inconclusive. Hughes-Schrader (1927) bred *I. purchasi* collected from various localities in the United States, Guatemala, and Italy; individual rather than geographic variation in sex ratio was obtained. Although Kuwana (1922) reared exclusively female broods in several successive generations, males were present in nature in the stock from which his experimental material originated. In Italy, where previous workers found almost exclusively female populations, Menozzi (1927) and Costantino (1938) report the sporadic occurrence of large numbers of males in certain years and areas. The evidence is thus negative, but the question remains open pending more sustained and extensive breeding experiments.

Only one other case of hermaphroditism, excluding the cytologically unknown phorid fly Termitoxenia, has been recorded among insects — this is the stonefly, *Perla marginata* (Junker, 1923), which presents a situation complimentary to *Icerya purchasi* — the differentiation in the male gonad of both male and female gametes. The ovary of the male Perla is nonfunctional; comparison with Icerya discloses two factors essential to successful operation of monoecious hermaphroditism. These are: (1) a regulation of the cytological mechanism of sex determination. Absent in Perla, this is achieved in *I. purchasi* by the gonial reduction which establishes the haplo-diploid sex mechanism of the iceryines within the hermaphroditic

gonad. And (2), correlated modification of the anatomy of the reproductive organs of one sex to permit liberation and functioning of the gametes of both sexes. This also is lacking in Perla and developed in *I. purchasi* where the process of spermiogenesis itself transforms the hermaphroditic gonad from a functional testis to a functional ovary. In considering the origin of hermaphroditism in *Icerya purchasi* we may safely assume that facultative arrhenotoky and male haploidy were already established in the Iceryini. A single mutation resulting in the reduction to the haploid condition of certain centrally located gonia in the female then might set in motion the already determined mechanisms of haploid male and diploid female meiosis within the single gonad (factor 1, above). Normal spatial and temporal differentiation of male and female gonia, each assuming the position and undergoing the maneuvers characteristic respectively of testis and ovary in true male and female, then automatically ensures an anatomically functional hermaphroditic gonad (factor 2, above).

Once established, hermaphroditism like parthenogenesis should have selective value in coccids where hazards of mating are extreme. Furthermore, if the true males are ever successful in fertilization, this should partially offset the decrease in recombination-variability resulting from a closed cycle of self fertilization. In this connection the question of the possible spread of hermaphroditism among Iceryini is of interest. The tribe comprises two groups (Table 1, page 130). Four of the five genera in group 2 are known on cytological grounds to constitute a closely knit assemblage identical in chromosome complement, facultative arrhenotoky, and male haploidy. Hermaphroditism, in the limits of present knowledge, is restricted to the genus Icerya, and within the genus *I. montserratensis*, *I. littoralis*, and *I. similis* show no trace of it. Recently however, two new and as yet undescribed species have been discovered in which gynomonoecious hermaphrodites have replaced females (Hughes-Schrader, unpub.). One of these stands taxonomically in close relation to both Icerya and Crypticerya (Morrison, personal communication). Further studies in both genera may well disclose a wider spread of the hermaphrodite habit than has been suspected heretofore. Group 1 of the Iceryini (Table 1) was erected for the single genus Gueriniella; here males are entirely unknown both in nature and in the extensive life history studies of Vayssière (1926). Ordinarily the assumption of thelyotoky would seem warranted. But in view of the functional hermaphroditism now known in three species of the tribe on the one hand, and on the other the close affinity on general taxonomic grounds of the iceryine genera, the possibility that Gueriniella is an hermaphroditic species which has lost the parthenogenetic potency deserves investigation.

C. *Polar Bodies in Development*

Still another unique modification of the female meiotic mechanism was discovered by Schrader (1923b) in *Pseudococcus citri* and *P. maritimus.* This involves the active participation of the polar bodies in development. Walczuch (1932) confirms his findings for *P. citri* and extends them to include *P. adonidum, P. nipae,* and *Trionymus diminutus.* The role of the polar bodies in development concerns the origin of the mycetocytes — those specialized cells which harbor the symbiotic fungi so widely distributed among insects, especially in Homoptera. The mutual adaptations of plant and animal organism are diverse and curious, as witness the case in hand.

In Pseudococcus the mycetocytes are organized into a definite organ, the mycetome. The symbionts are transmitted from one generation to the next through the eggs, each of which receives a number of fungi from the mycetome of the mother. In transfer from mycetome to egg, the symbionts are dissociated from the mycetocytes and enter the egg "naked." During the early development of the egg they are again picked up by definite cells of the embryo, cells which thus become the mycetocytes and give rise to the mycetome organ of the adult. Schrader's hypothesis of the origin of the mycetocytes was based on the numerical relations of chromosome counts in different generations of the nuclei involved in mycetome development, and was proved by a cytological confirmation, step by step, of the events postulated. Taking *P. citri* (2n = 10) as an example, the origin of mycetocytes in Pseudococcus is as follows: A polar nucleus is formed by the fusion of the first and second polar bodies. This contains 15 chromosomes (10 from the first plus 5 from the second polar body), since the chromatids of the 5 dyads of the first polar body disjoin at first telophase. The polar nucleus then divides, usually three times, giving rise to polar derivatives each with 15 chromosomes. These then fuse, either in pairs, giving 30-chromosome nuclei, or with one or two cleavage cells producing nuclei with 25 and 35 chromosomes. The resultant giant cells may undergo endomitosis giving rise to nuclei with circa 60–70 chromosomes. At this stage the giant cells migrate to and engulf the symbionts, thus becoming the primary mycetocytes which in turn give rise to the mycetome organ of the adult. Multiplication of chromosomes endomitotically may continue in the mycetocytes; an upper limit of approximately 200 is reported by Buchner (1921), Schrader (1923b) and Walczuch (1932). (Chromosome complements of cleavage nucleus, polar nucleus, polar derivative, and giant cells are shown in fig. 12, *a–f*.)

Another aspect of the fusion process deserves mention. Since mitosis in the polar body derivatives is not synchronous with that in cleavage cells, the nuclei involved in a given fusion are frequently in different phases

of the mitotic cycle. Metaphase plate formation is then delayed until all the sets of chromosomes have become condensed. Different coiling cycles in different chromosome sets within a single nucleus are by no means confined to coccids, but the phenomenon may have an especial significance here with reference to the evolution of asynapsis in male meiosis and the sex determining mechanism in this group of coccids (compare page 183).

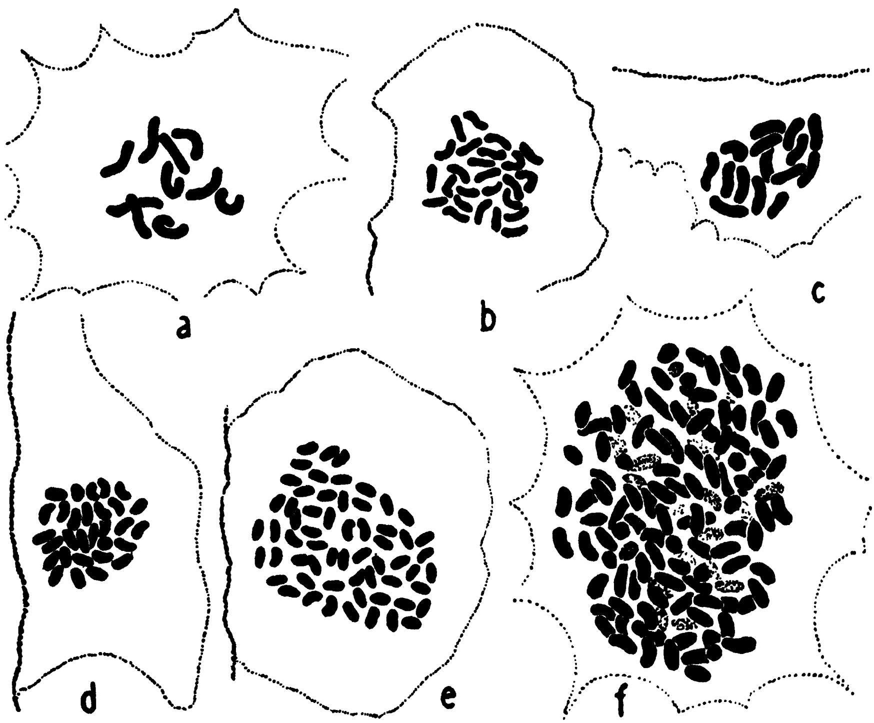


FIG. 12

Origin of mycetocytes; *Pseudococcus citri*. *a*, cleavage nucleus, 10 chromosomes; *b*, giant cell, 25 chromosomes from fusion of one polar nucleus derivative (15) and one cleavage nucleus (10); *c*, polar nucleus, 15 chromosomes = 5 + 5 from first polar body + 5 from second polar body; *d*, giant cell, 30 chromosomes from fusion of 2 polar nucleus derivatives; *e*, endomitotic giant cell, 60 chromosomes; *f*, mycetocyte nucleus from adult, ca 200 chromosomes. From Schrader (1923b).

It is of interest also that the impetus to fusion stems from the polar body derivatives; cleavage nuclei coming into contact with one another, even in the same region of the egg, do not fuse. This may be viewed as a precocious development (and consequent extension to the polar bodies) of those conditions or properties, whatever they may be, of the female pronucleus which induce its fusion with the male nucleus. A similar modifi-

cation, but initiated at interkinesis and hence restricted to the second polar body, may underlie the "self-fertilization" of the female pronucleus by the second polar body in eggs of the facultatively parthenogenetic race of various Lecanium species (compare page 143).

V. Specialized Types of Male Meiosis

Diversity and bizarrerie characterize the evolution of the meiotic mechanism in the male line. And the end is not yet, since only a few of the existing groups have been sampled. In the Diaspidoïdae, largest and most specialized of the three major sections of the Coccoïdae (Table 1), the cytology of males is unknown. In the Lecanoïdae but 3 of 10 families have been studied. One of the two families of the Margaroïdae is unknown; of the other, each of the 3 tribes investigated discloses a uniquely specialized male meiosis, while 12 tribes remain unexplored!

The 23 species for which data on male meiosis are available fall into 5 categories. One, retaining a primitive meiosis and represented by Puto (family Eriococcidae), has already been reviewed. In the second, also represented by a single species, *Matsucoccus gallicola* (family Margarodidae), a compound sex chromosome mechanism has evolved in an otherwise primitive meiosis (page 190). The remaining three types are sharply differentiated and highly specialized. They are represented by (1) 7 species of the Iceryini, (2) 4 species of Llaveiini (both tribes of the family Margarodidae), and (3) 9 species among 3 families of lecanoïds (Eriococcidae, Lecaniidae, and Dactylopiidae). These diversely specialized types do not stand in any simple or direct evolutionary sequence. However, the persistence in at least two species of a primitive meiosis, and the recurrence in diverse groups of similar changes variously developed and combined, lend hope that annectent forms may still exist which will disclose the inter-relationships of the now apparently discontinuous types.

A. *The Iceryine Type*

1. Meiosis

As already noted, the males of all iceryine coccids thus far studied are haploid. The point of major interest in their meiosis is thus its adaptation to haploidy — how it has become regulated for the production of normal haploid gametes. Spermatogenesis in the haploid iceryine was first described in the true males of *Icerya purchasi* (Schrader and Hughes-Schrader, 1926); its course is identical in the haploid testicular tissue of the *I. purchasi* hermaphrodites (Hughes-Schrader, 1927), in the males of *I. montserratensis*, *I. littoralis*, *Echinicerya anomala*, *Crypticerya rosae*, (Hughes-Schrader, 1930a), and *Steatococcus tuberculatus* (Hughes-Schrader and Ris, 1941). In all, the chromosome number is 2, and the chromosome

cycle is unusually open to cytological analysis. Meiosis comprises a single division, demonstrably equational in character. Its meiotic nature is evident in the prolonged prophase, and in the extreme condensation of the

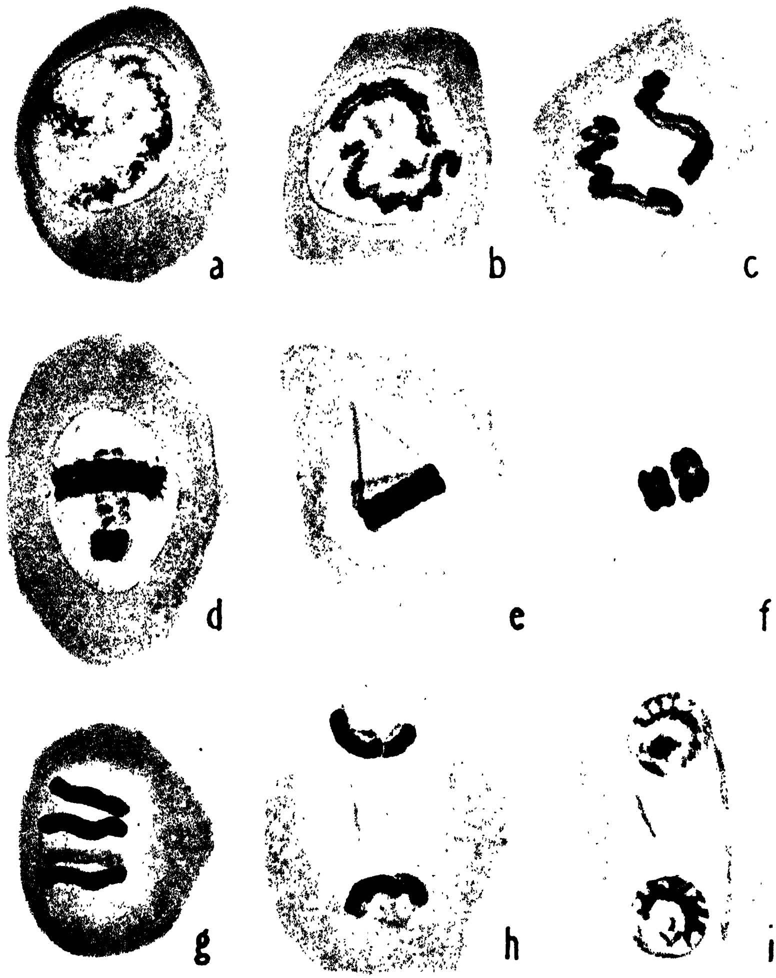


FIG. 13

Meiosis in haploid male. *a, b, c, g,* and *i* from *Icerya purchasi; d, e, f, h,* from *Stealococcus tuberculatus. a–c,* prophase with increasing separation of chromatids in the two univalents; *d,* late prophase, incipient separation of half-chromatids visible in end view of lower chromosome; *e,* metaphase, chromosome viewed from side; diffuse kinetochore and sheet of chromosomal fibers; *f,* metaphase chromosomes viewed from end–quadripartite; *g,* parallel anaphasic disjunction; *h,* bending of chromosomes toward pole in late anaphase; *i,* binucleate spermatid. *a, b, c, g,* and *i* from Hughes-Schrader (1927).

metaphase chromosomes. That only one division occurs is demonstrable not only through a close seriation of stages from final spermatogonium to mature sperm (fig. 13, *a–i*), but receives further confirmation in the fact that cross sections of the bundles formed by the sperm of each cyst invariably show 32 sperm per bundle, while the number of nuclei per cyst at final spermatogonial telophase and at meiotic metaphase is as invariably 16. Meiosis is thus seen to be completely stabilized and regulated to the haploid condition; it now comprises but a single division which is close to somatic in type.

Of interest also in the iceryine meiotic division is the intra-nuclear origin of the spindle. Its poles are formed by opposing conical projections of the nuclear membrane in which the triangular sheets of chromosomal spindle fibers are focussed (fig. 13, *e*). The membrane persists at least throughout metaphase (Icerya) and often even until late anaphase (Steatococcus). In these forms therefore there can be no doubt of the exclusively nuclear origin of the spindle.

Incomplete cytoplasmic division, followed by a re-fusion of the derivatives of each primary spermatocyte to form quadrinucleate spermatids was noted as a constant feature of the primitive type of male meiosis exemplified by Puto. In the iceryines a further development of this character has occurred; the cytoplasmic cleavage is entirely suppressed and the spermatids are binucleate from telophase on.

2. Spermiogenesis

Thanks to its unique origin and structure the iceryine sperm affords direct observational evidence on the chromosome cycle of spermiogenesis and the orientation of chromosomes in the mature sperm — stages not ordinarily open to chromosomal analysis (Hughes-Schrader, 1946).

The iceryine sperm is formed by the invasion of the cytoplasmic anlage of the spermatid tail by a delicate nuclear diverticulum containing the chromosomes extended in linear order and attached end to end. On completion of the migration of chromosomes into the tail the latter breaks free from the rest of the cell and is transformed directly into the functional sperm. The spermatid cell body including the nucleus, apparently intact but now empty of chromosomes, and all formed cytoplasmic constituents are discarded and eventually engulfed by the sheath cells of the sperm bundles.

In actual appearance the migration of the chromosomes out of the nucleus into the tail of the spermatid is seen as the progressive elongation of a delicate Feulgen positive filament continuous with the chromatin net of the nucleus (fig. 14). This filament is differentiated by a constriction into two regions of different lengths, the shorter one always in advance.

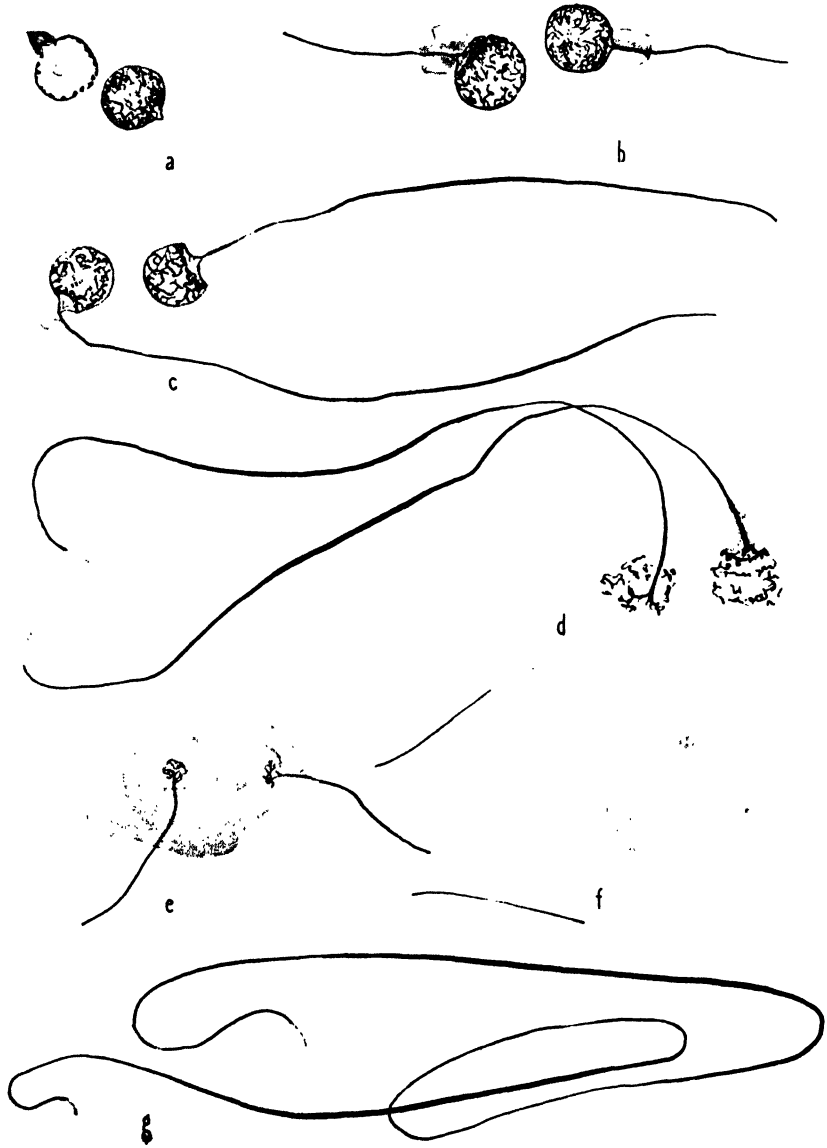


FIG. 14. Sperm formation in Iceryini. Chromosomes (Feulgen-positive) drawn in ink, balance (fast green) in water color. *a*, early spermatid, nucleus at left in optical section, at right in surface view; cytoplasmic anlage of tail; *b*, invasion of tail by nuclear diverticulum containing a chromosome; *c*, migration into tail of first chromosome completed; *d*, start of migration of second chromosome, correlated diminution of nuclear chromosomal material; *e*, later stage of same; *f*, nuclei completely empty of chromosomes; (only proximal end of tail shown in *e* and *f*); *g*, mature sperm with 2 chromosomes in linear alignment; nuclei discarded with cell body. From Hughes-Schrader (1946).

Concomitant with the elongation of the tail the chromatin net of the nucleus is progressively reduced until on the completion of tail formation the nucleus is completely Feulgen negative. The structure of the tail is demonstrable after swelling in aceto-orcein; it comprises the Feulgen-positive filament enveloped in a tubular outgrowth of nuclear membrane and a delicate cytoplasmic sheath. The possibility that the centriole (possibly embedded in the nuclear membrane at the meiotic division) may determine the point of outgrowth of the nuclear diverticulum cannot be excluded, although no centriole is demonstrable in spermatid or male pronucleus.

The identification of the two sections of the chromatin filament of the tail as the two chromosomes of the spermatid nucleus rests on the following facts: During its formation the chromatin filament of the tail is directly continuous with the chromatin net of the nucleus — it grows at the expense of the latter, and finally contains in itself all the chromatin of the nucleus; it is moreover the only nuclear derivative to be carried into the egg at fertilization. Within the egg the male pronucleus is formed by contraction and vesiculation of the tail filament and this pronucleus gives rise to the two chromosomes of the haploid set. Finally, the relative size of the two regions of the tail filament corresponds with that of the chromosomes, when allowance is made for their asynchronous spiralization during spermiogenesis.

A complex spiralization cycle is associated with spermiogenesis. Four successive phases are distinguishable either by direct observation or as expressed by changes in diameter and length of the chromosome: (1) despiralization in the spermatid telophase chromosome to the resting condition; (2) contraction through spiralization while the chromosomes are moving into the tail; (3) a second period of elongation or uncoiling coextensive with the growth of the sperm to its maximum length; and (4) a final period of spiralization. This last phase concludes with the two chromosomes still in a relatively extended condition — respectively 11 and 13 times as long as in meiotic metaphase.

The two values just cited characterize respectively the shorter and longer of the two chromosomes. The difference results from the asynchrony of the two chromosomes throughout the postmeiotic cycle. The shorter chromosome invariably leads the way in the migration out of the nucleus, and maintains this precocity through all the following changes. Synchrony is re-established by the time the chromosomes of the male pronucleus participate in cleavage mitosis.

For their general cytological significance certain features of this unique spermiogenesis deserve further emphasis. (1) Localization of special properties in chromosome ends is shown not only in the initiation of chromo-

some migration by one end of a particular chromosome, but also in the tenacious terminal association of the two chromosomes throughout spermiogenesis. (2) Clearly demonstrated also is the potential mobility of chromosomes while in a highly extended condition, and independent of the action of spindle fibers. (3) The complex postmeiotic spiralization cycle may well prove to be a fundamental feature in spermiogenesis in general. Its first phase — telophasic despiralization in the spermatid — is practically universal. In the tipulid fly *Dicranomyia trinotata* (Wolf, 1939) — the only other case in which later stages have proved analyzable — a spiralization cycle identical with the first three phases of that of the iceryines is demonstrable before the density of the sperm head precludes further observation. (4) Concerning the orientation of chromosomes in the mature sperm the forms now open to analysis disclose two patterns — linear alignment correlated with terminal association in the iceryines, and the persistence, at least until the period of elongation of the nucleus, of the telophase orientation in Dicranomyia. No generalization is as yet warranted.

The type of spermiogenesis just described occurs in all members of the tribe Iceryini in which these stages have been studied — *Echinicerya anomala*, *Steatococcus tuberculatus*, *Icerya purchasi* (Hughes-Schrader, 1946), and in an undescribed species probably representative of a new genus (Hughes-Schrader, unpub.). Whether it antedates tribal differentiation is not known, since adequate data on spermiogenesis are as yet available in no other group.

B. *The Llaveiine Type*

The Llaveiini, a second tribe of the monophlebine margarodids to which the Iceryini also belong, have remained in comparison with the latter far more primitive in reproductive processes. Neither parthenogenesis nor hermaphroditism occurs, female meiosis is orthodox, the sex ratio approaches equality, and the ancestral sex-chromosome mechanism is retained. Meiosis in the male line has however undergone an extensive evolution in four major areas of specialization: (1) vesiculation of the prophase nucleus, (2) the spindle apparatus, (3) development and regulation of asynapsis, and (4) autonomy of chromosome constituents, — chromatids, half-chromatids, etc., in certain of the mitotic processes. Representatives of all three genera now included in the tribe — Llaveia, Llaveiella, and Protortonia — have been studied cytologically (Schrader, 1931; Hughes-Schrader, 1931, 1940, 1942), and in addition some data are available on five other species as yet undescribed (Hughes-Schrader, unpub.). The genus *Nautococcus*, formerly referred to the Llaveiini, has recently been reclassified as a member of the tribe Coelostomidiini in the

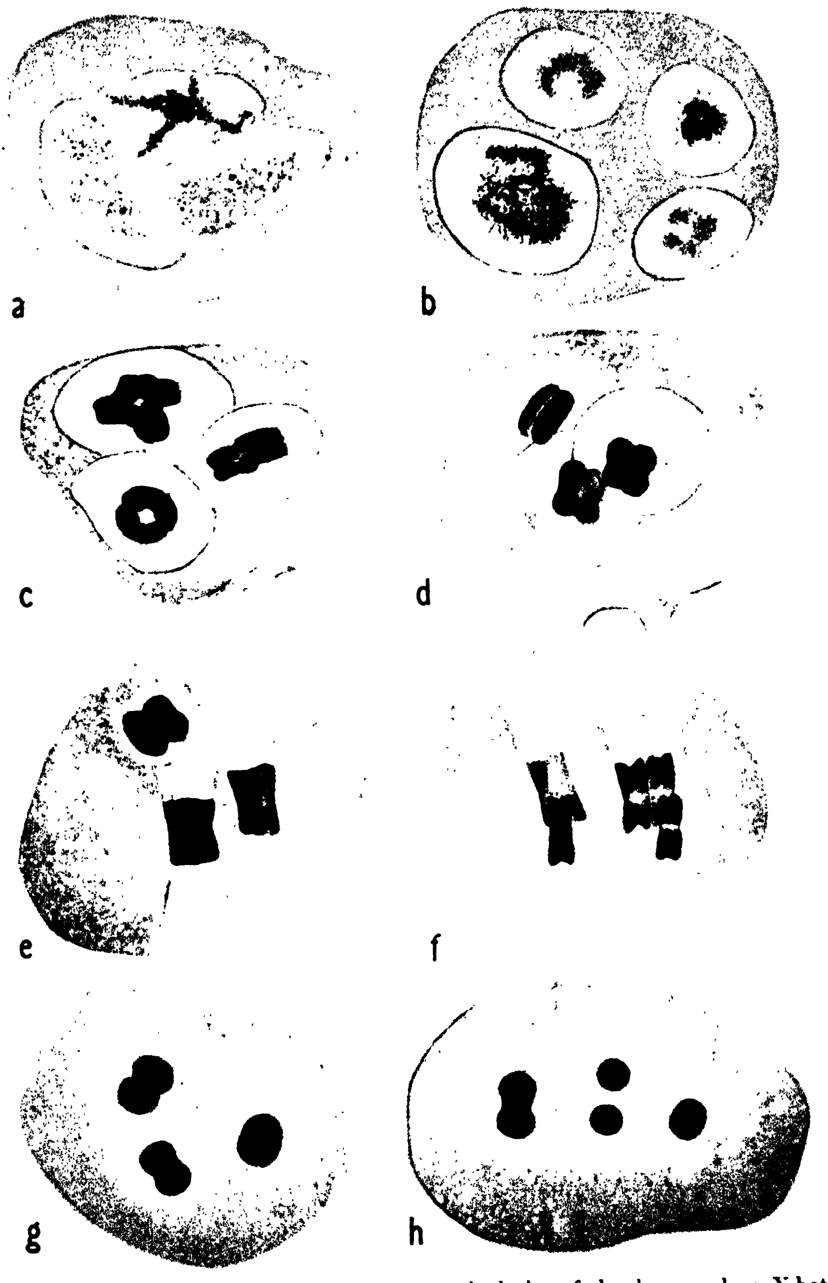


FIG. 15. Meiosis, male *Llaveia bouvari*. *a*, vesiculation of phophase nucleus, X hetero chromatic; *b*, vesiculation, with shorter pair of autosomes asynaptic and in separate vesicles; *c*, *d*, diakinesis; *e*, formation of chromosomal fibers; *f*, metaphase I, asynaptic autosomes dividing independently; *g*, typical metaphase I; *h*, metaphase I with asynaptic pair, polar view. From Hughes-Schrader (1931).

related subfamily Coelostomidiinae of the same family Margarodidae. Cytologically, however, *Nautococcus* is a typical llaveiine and must be considered here in relation to this group (compare page 196). Of these four genera Llaveia is cytologically the least specialized. Llaveiella, Nautococcus, and those three of the new species for which male meiotic material is available each show characteristic developments in one or more of the three major lines enumerated above but are still clearly closely akin to Llaveia, while Protortonia stands out as the most highly evolved in all areas of specialization. The chromosome number is the same in all — the diploid complement of the female comprises 2 pairs of autosomes plus 2 sex chromosomes, that of the male 2 pairs of autosomes plus 1 X chromosome. The following resumé of male meiosis in Llaveia is based on *L. bouvari*, (Hughes-Schrader, 1931) and *L. oaxacoensis* (Hughes-Schrader, unpub.). The remaining forms will then be considered only in relation to differential developments.

1. Llaveia

Meiotic specialization is initiated by a lobulation of the prophase nucleus into three vesicles. One of these contains the precociously condensed X chromosome, and in each of the others one autosomal bivalent develops (fig. 15, *a*, *c*). From the latter fact it follows that some degree of chromosome pairing precedes vesiculation. The precise nature of the pairing cannot be ascertained since the chromosomes are "unfixable" at the time of vesicle formation. They emerge from the diffuse condition already in diakinetic form and it can then be seen that in the majority of cases normal bivalents are formed (fig. 15, *c*, *d*). The structure and metaphase orientation of the bivalents correspond closely with those of Nautococcus and will be considered in connection with the latter which are more open to analysis.

In some 5 per cent of the spermatocytes of Llaveia the shorter pair of autosomes is completely asynaptic. This first becomes evident in the prophase vesiculation, for each asynaptic chromosome lies in a separate vesicle or, more rarely, a separate lobe of one vesicle (fig. 15, *b*). No meiotic irregularity follows asynapsis. The asynaptic chromosomes divide independently in the first division (fig. 15, *f*, *h*); at interkinesis their derivative chromatids reassociate and segregate normally in the second division. The third area of specialization lies in the unique meiotic division figure. In prometaphase the vesicle walls disappear. From the non-conjunctive side of each chromatid there then develops an outward flaring mass of chromosomal fibers (fig. 15, *e*). When the two halves of a bivalent are closely appressed together the half spindle components of the two chromatids appear as a single megaphone-shaped mass, whose dual structure

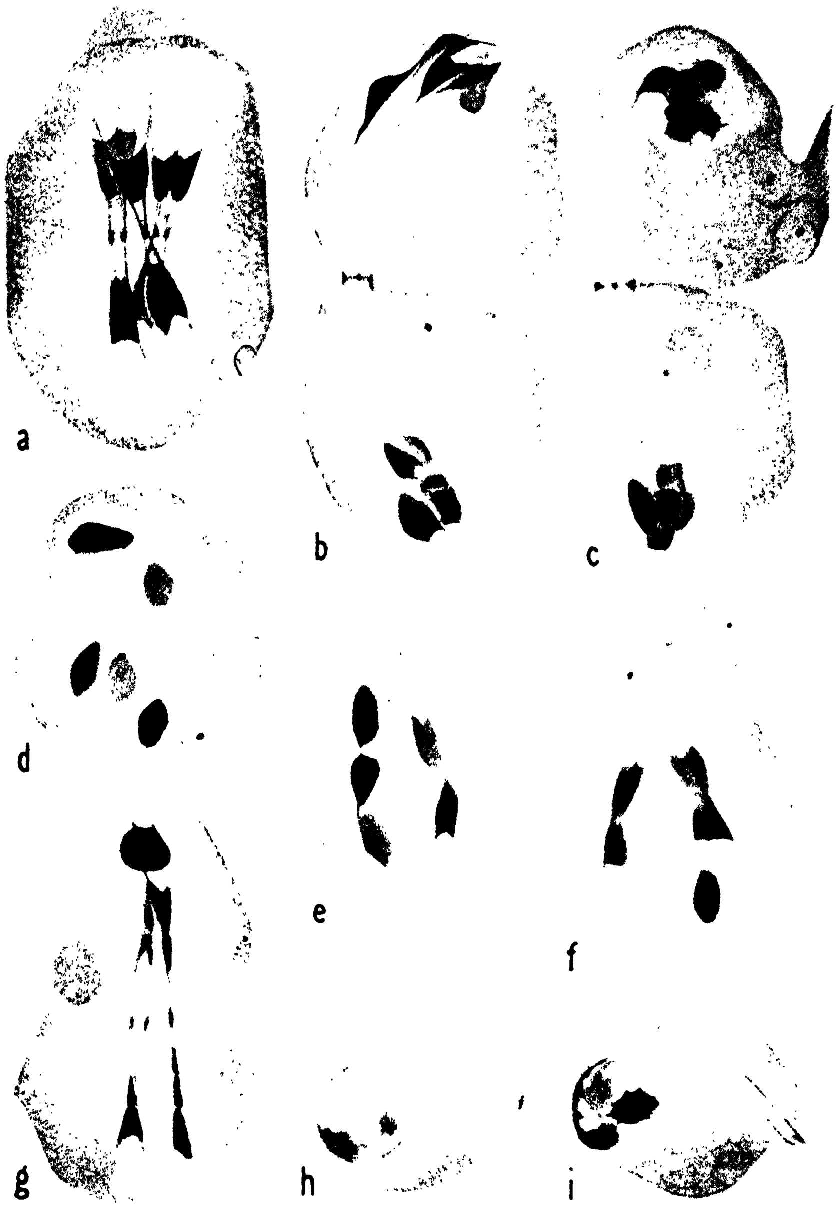


FIG. 16

Meiosis, *Llaveia bouvari*, continued. *a*, anaphase I; independent chromosomal spindle units; *b*, and *c*, fusion of interzonals into stembody; *d*, chromatid disjunction in interkinesis; *e*, secondary pairing; *f*, metaphase II; *g*, anaphase II; *h*, *i*, spermatids. From Hughes-Schrader (1931).

is however always apparent in anaphase. When the chromosomal fibers first form they are oriented solely with reference to the chromosome. The individual chromosomal spindle units resulting thus evince at first no common orientation. Only later, and gradually, do they swing into parallel alignment and thus establish a bipolar figure which is compounded of units each of whose chromosomal fibers diverge distally instead of converging to a common center. The chromosomal elements now become deformed by elongation in the long axis of the spindle unit. Interchromatid constrictions are eliminated, and the distal surface of each chromatid becomes cusped as though its substance were drawn out peripherally in a delicate flange along the boundary between cytoplasm and chromosomal fibers (compare *e* and *f*, fig. 15).

At anaphase a tubular interzonal connective (fig. 16, *a*), which in cross section shows a distinct delimiting sheath, develops between each two disjoining chromatids. The chromosomal fibers shorten and fade out distally; the interzonals become progressively more elongate and slender. Finally, as the cytoplasmic cleavage furrow cuts in, the separate interzonal tubes are brought together centrally and fuse into a single stem body whose continued elongation is evident in Figure 16, *b* and *c*.

Interkinesis witnesses a complete disjunction of the chromatids, often with wide spatial separation (fig. 16, *d*). Since little or no uncoiling takes place the chromatids remain constantly identifiable by their size. It is thus possible to see that the ensuing reassociation in pairs of the autosomal chromatids is in fact a secondary pairing of homologues. Since the reassociation was found to be invariable, in several hundred second spermatocytes checked, it follows as noted above that the asynaptically developed chromatids also undergo secondary pairing; their behavior thereafter is indistinguishable from dyads derived from bivalents. The persistence of secondary pairing, even after suppression of synapsis, thus serves to stabilize asynapsis.

Spindle formation in the second division parallels that of the first with the single exception of the passivity of the X chromosome. The latter gives rise to no spindle fibers, is caught up in the chromosomal fibers of one of the autosomes, and is moved with it to one pole (fig. 16, *g*). Deformation of chromatids in the individual spindle units is even more marked than in the first division. The plasticity of the chromosome, here particularly evident, is further considered on page 189.

As in other coccids, quadrinucleate spermatids are formed by the fusion of the derivatives of each primary spermatocyte. The regulation of this process by the persistence through both meiotic divisions of the undivided cytoplasmic stalk of the primary spermatocyte can be demonstrated in the llaveiines.

The unique specializations of the male Llaveia meiosis may now be summarized as follows: (1) Vesiculation of the prophase nucleus with resultant isolation of each bivalent or univalent in a separate vesicle. (2) Spindle with broadly dispersed poles, compounded of precociously formed individual spindle units, each with divergent, nonfocussed chromosomal fibers and tubular anaphasic interzonal connective; origin of chromosomal fibers prior to establishment of bipolarity; fusion of interzonals to form a stem body. (3) Asynapsis, in a small proportion of the spermatocytes, of the shorter pair of autosomes, which divide independently in the first — and segregate after a secondary pairing at interkinesis — in the second division. Not uniquely llaveiine, but a part of the primitive coccid heritage, are the disjunction of chromatids at first telophase, secondary pairing in interkinesis, absence of continuous fibers in the spindle, and fusion of spermatids.

2. Nautococcus

In *Nautococcus schraderae* (Hughes-Schrader, 1942) the meiotic chromosomal fibers develop precociously while the long axis of the chromosome is still identifiable. The orientation of the bivalents at first metaphase — masked in Llaveia by the contraction of the chromatids to spherical form prior to the appearance of spindle fibers — can thus be determined (compare Ris, 1942, p. 312). Upon resolution of the chiasmata in late diakinesis the chromosomes assume an end to end position (fig. 17, 1). No terminal adhesion ensues, however, and the two chromosomes may shift position relative to each other while maintaining identical orientation toward the poles. Chromosomal fibers then form along the length of each chromatid and, with the progressive contraction of the chromosome, become concentrated into the typical megaphone shape characteristic of the llaveiine meiotic chromosome (fig. 17, 1). Orientation for first metaphase then brings the secondary (inter-chromatid) split into the equatorial plane. This independent as opposed to co-orientation of the two chromosomes of the bivalent makes the first division equational for non-crossover regions. The bivalents of Llaveia and Llaveiella (fig. 17, 2, 3) have the same basic structure; the primary split may be identified there, as in Nautococcus, by its greater width as compared with the secondary, and it is always found to be parallel to the inter-polar axis. It is safe to conclude that in the llaveiines metaphase orientation and the sequence of equational and reductional separation (disregarding crossing over) conforms to the basic coccid type of meiosis (p. 137). The precocity of chromosomal fiber formation also shows that the meiotic chromosome obeys the laws of behavior imposed by the diffuse kinetochore: The whole chromosome is oriented in the equatorial plane at metaphase, and chromosomal fibers form along the

whole poleward surface of each chromatid. These relations are masked in the other llaveiines and in later stages of Nautococcus by the extensive deformation of the chromatids associated with the formation and elongation of the spindle elements.

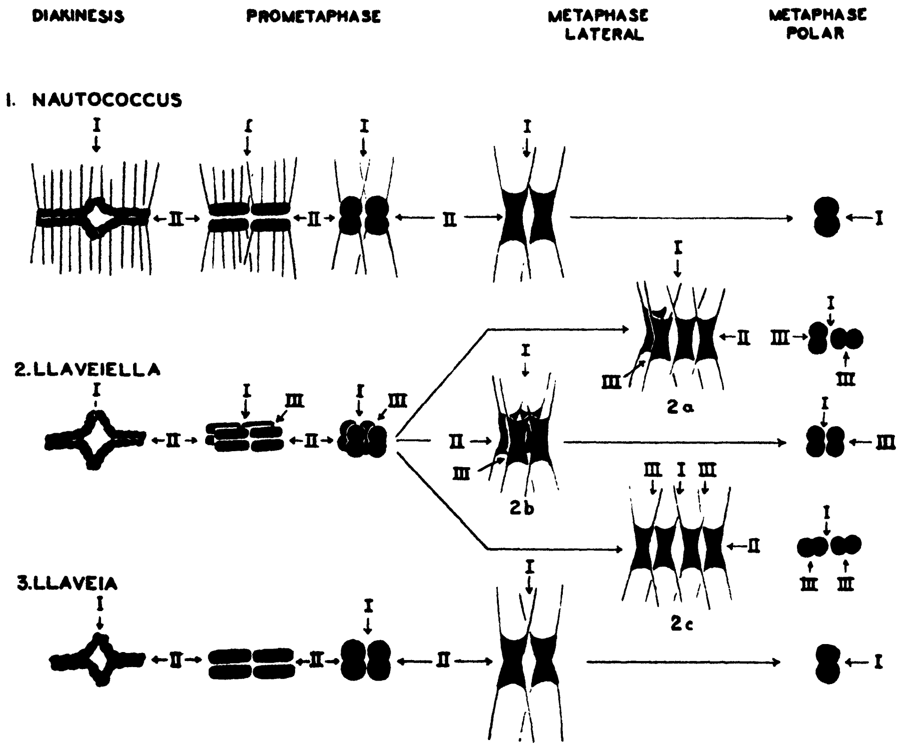


FIG. 17

Diagram of bivalent structure in male Llaveiini. 1. Nautococcus; homologous chromosomes assume end to end position; precocity of chromosomal fibers permits analysis of orientation of bivalent at first metaphase I = primary split (junction of ends of homologues); II = secondary (inter-chromatid) split, utilized in first division. 2. Llaveiella; identity of bivalent structure with 1 permits identification of splits I and II. Homologous chromosomes may shift position at metaphase, as in 2a, 2b, 2c. 3. Llaveia; bivalent structure identical with Nautococcus.

Anaphasic disjunction in the two chromosomes of a bivalent is almost always asynchronous in the first division of Nautococcus. The asynchrony is variable in expression. The cones of chromosomal fibers and the interzonal connectives may remain distinct (fig. 18, *a*). Or, if one pair of chromatids is far in advance of the other, the two individual spindle units may fuse and a linear aggregate of four chromatids result (fig. 18, *b*). As a result the two chromatids are brought into telophase already in the

parallel alignment requisite for their segregation in the second division (fig. 18, *d*). The dyads thus formed undergo no disjunction in interkinesis. If on the other hand no linear aggregate be formed, the chromatids become widely separated in the interkinetic nucleus (fig. 18, *e*, *f*) and the usual



a b c d e f

FIG. 18

Nautococcus schraderi. *a*, spermatocyte, metaphase I, with asynchronous disjunction in the two chromosomes of each bivalent; *b*, fusion of asynchronous anaphasic spindle units (right hand bivalent) to form linear aggregate; *c*, telophase I after partial, and *d*, after complete fusion of spindle units — no disjunction of chromatids follows; *e*, telophase I with disjunction of chromatids; *f*, secondary pairing in interkinesis. From Hughes-Schrader (1942).

secondary pairing then ensues. In other words fusion of the two asynchronous anaphase spindle units of a bivalent brings about a precocious disjunction and realignment.

N. schraderae is further distinguished by the presence in a considerable proportion of the individuals of from one to five supernumerary chromosomes, varying as to presence and number within the individual. Elimination of supernumeraries is frequent in embryos and may occur as late

as the last spermatogonial division. In male meiosis they show no pairing, divide normally in the first and are passively distributed at random in the second division. The possible origin of these supernumeraries through fragmentation of an ancestral X chromosome is considered on page 191.

In respect to other lines of specialization developed in Llaveia, Nautococcus presents a less differentiated condition. Asynapsis, again of the shorter pair of autosomes, occurs in a smaller percentage of the spermatocytes. It is similarly compatible with regular segregation through the operation of secondary pairing at interkinesis. Again, the early meiotic prophase nucleus becomes lobulated, each bivalent and univalent passing through the later prophase stages in more or less well defined lobes; an incomplete expression of the tendency toward the vesicle formation found in all the other llaveiines is suggested.

3. Llaveiella

Additional specializations superimposed on the meiotic pattern of Llaveia and Nautococcus are encountered in *Llaveiella taenechina* (Hughes-Schrader, 1942). These are variably expressed, to different degrees and in different combinations in the cells of the same testis, while a small proportion of the spermatocytes adheres strictly to the Llaveia pattern.

Most striking, and most completely expressed since it occurs in the majority of the spermatocytes, is the complete cleavage of the meiotic chromosomes along the tertiary split, brought about by the separation of half chromatid coils. The 4 strand structure of the metaphase chromosome is thus unequivocally demonstrated. An even higher valence is probable, since further separation of the constituent coils of the half chromatid (quaternary split) is occasionally visible at second anaphase (fig. 19, *g*). Quarter chromatids may then be spatially distinct, indicating an 8 strand structure in the first meiotic metaphase chromosome.

The two chromosomes of a bivalent may shift position relative to each other while maintaining an identical orientation to the poles. The resultant variation in bivalent structure is shown in Figure 17, 2. If the end to end alignment of the component chromosomes is preserved the bivalent appears four parted whether viewed from frontal or end aspect of the chromosomes (fig. 17, 2*b*). The spacing between pairs of half chromatids is, however, wider in the frontal aspect, where it represents the primary split, than in the end view where the tertiary split is visible. If the 2 chromosomes shift so as to lie at right angles to each other, 6 half chromatids and all three splits are visible from one side, 4 chromatids from the other (fig. 17, 2*a*). If the 2 chromosomes come to stand parallel, all 8 elements are visible when the ends of both chromosomes are presented to the eye of the observer (fig. 17, 2*c*). Polar views all show 4 half chro-

matids in the various spatial relations shown at extreme right in Figure 17, 2.

Mitotic independence of the multiple elements of the chromosome is further evident in the anaphase, for here the pairs of half chromatids

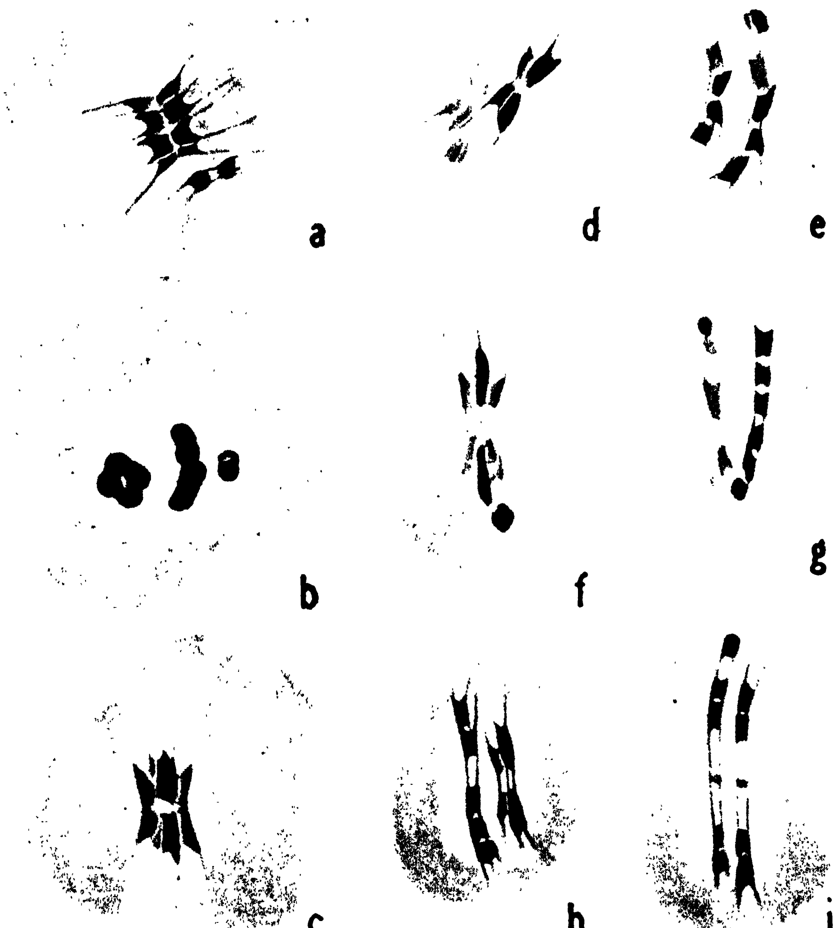


FIG. 19

Llaveiella taenechina. *a*, primary spermatocyte in metaphase I; component chromosomes shifted so that 4 parted end view of each is seen; *b*, polar aspect metaphase I; right hand bivalent as in *a*, left as in *c*; *c*, asynchrony in disjunction in pairs of half chromatids (one bivalent only drawn); *d*, *e*, *f*, anaphase II — asynchronous disjunction of half chromatids separated by tertiary split; *g*, *h*, *i*, fusion of asynchronous spindle units into linear aggregates; quaternary split in right hand unit of *g*. From Hughes-Schrader (1940).

separated by the tertiary split may disjoin independently. The asynchrony at first anaphase is similar to but slighter in degree than that met in Nautococcus. In Llaveiella, however, a pair of half chromatids instead of whole chromatids forms the unit in the anaphasic movement (fig. 19, *c*).

But it is in the second anaphase that the autonomy of chromosome elements reaches a really bizarre expression (fig. 19, *d–i*). Here in the great majority of secondary spermatocytes each pair of half chromatids forms its own spindle unit and its members disjoin at different times and rates. Three factors affecting the resultant anaphase configuration are observable. One is the progressive deformation of the chromatid in the elongating and narrowing spindle unit. The second is the formation and elongation of the tubular interzonal connective. Sometimes one, sometimes the other, is first apparent, and both may be expressed to different degrees in the two spindle units of a single dyad (fig. 19, *d*, *f*, *h*). The third factor lies in the degree of fusion undergone by the spindle units. Fusion of the two interzonals of a dyad is general. If the members of one pair of half chromatids are far in advance of the other complete fusion usually follows resulting in a linear aggregate of 4 half chromatids in a single spindle unit. If the X is caught up in the chromosomal fibers of such a unit, an aggregate of 5 elements results (fig. 19, *h*, *i*). The significance of these linear aggregates for an analysis of the division figure in Protortonia will be discussed in connection with the latter.

An extreme expression of chromatid autonomy is seen in the occasional complete dissociation of the chromatids of the X chromosome. In such cases the two chromatids pass through the prophase in separate vesicles and divide independently in the first division. The homologue of the tertiary split of the autosomal bivalent is here actually effective in division. That the two X chromatids reassociate at interkinesis and pass together to one pole in the second division follows from the fact that in several hundred second anaphases checked the X chromosome, even when clearly double, always acts as a single unit. Since fragments of chromosomes with diffuse kinetochores are capable of mitosis the question should be raised whether the dissociated X chromatids may not be the products of fragmentation. Opposed are the facts that the separated parts of the X are not seen in the soma but only at meiosis when an extreme and general separation of sister chromonemata is taking place in this species; that they are always half the size of the X and appear identical in all the males studied; and that reassociation of fragments, barring considerable previous duplication, is unlikely. Furthermore, in the related genus Protortonia the chromatids of the X are regularly dissociated in meiotic prophase. This point carries the more weight if we consider how the basic changes which have molded llaveiine meiosis occur repeatedly in different species

but vary with the species from an incipient expression in a small proportion of the cells to complete expression in all. The weight of evidence favors the original interpretation of autonomy of dissociated chromatids.

Another feature of general cytological interest associated with the subdivision of the chromosome lies in the behavior of the chromatids in the spermatid nucleus. In early second telophase nuclei the two sister chromatids of each autosome are well separated and undergoing progressive uncoiling; later stages show their reassociation in a joint coil. Visible here is a process which must occur when optically double chromosomes of a last spermatogonial telophase reappear in leptotene optically single. It is paralleled again in the secondary pairing of chromatids during interkinesis in male meiosis of aphids, when two chromatids which enter the resting phase in end to end association emerge from it parallel and jointly coiled (Ris, 1942).

The regulated asynapsis of the shorter pair of autosomes developed in Llaveia and Nautococcus occurs in a slightly larger percentage of the spermatocytes of Llaveiella. Rarely the longer pair is also asynaptic, and in a few instances asynapsis of both pairs in the same cell has been observed — the condition normal for Protortonia.

4. Protortonia

The most highly differentiated condition relative to each major line of evolution in llaveiine meiosis, is found in *Protortonia primitiva* (Schrader, 1931). Its peculiar specializations are summarized below.

In the transition to meiotic prophase the 5 condensed chromosomes of the last spermatagonial telophase appear as more and more diffuse masses whose number increases to 7 or 8 before progressive uncoiling makes them no longer countable (fig. 20, *a*, *b*, *c*). Concomitantly the nucleus is subdivided by successive budding into 4 vesicles, — one small, two medium, and one large. Four chromosomal elements condense within the large vesicle and 2 in each of the others; their subsequent history shows them to be the 10 dissociated chromatids of the 5 chromosomes of the male set (fig. 20, *d*, *e*). The X chromatids alone are identifiable, by their small size and negative heterochromacy. They occupy always the smallest vesicle; it seems safe to conclude therefrom and from the close correspondence with vesicle formation in the other llaveiines that the four chromatids in the large vesicle stem from one pair of autosomes while the two chromatids of one member of the second pair evolve in each of the other, medium-sized vesicles. The disjunction of chromatids must take place during the transition from gonial telophase to meiotic prophase and is probably indicated by the increase in the number of chromocenters observable at that time.

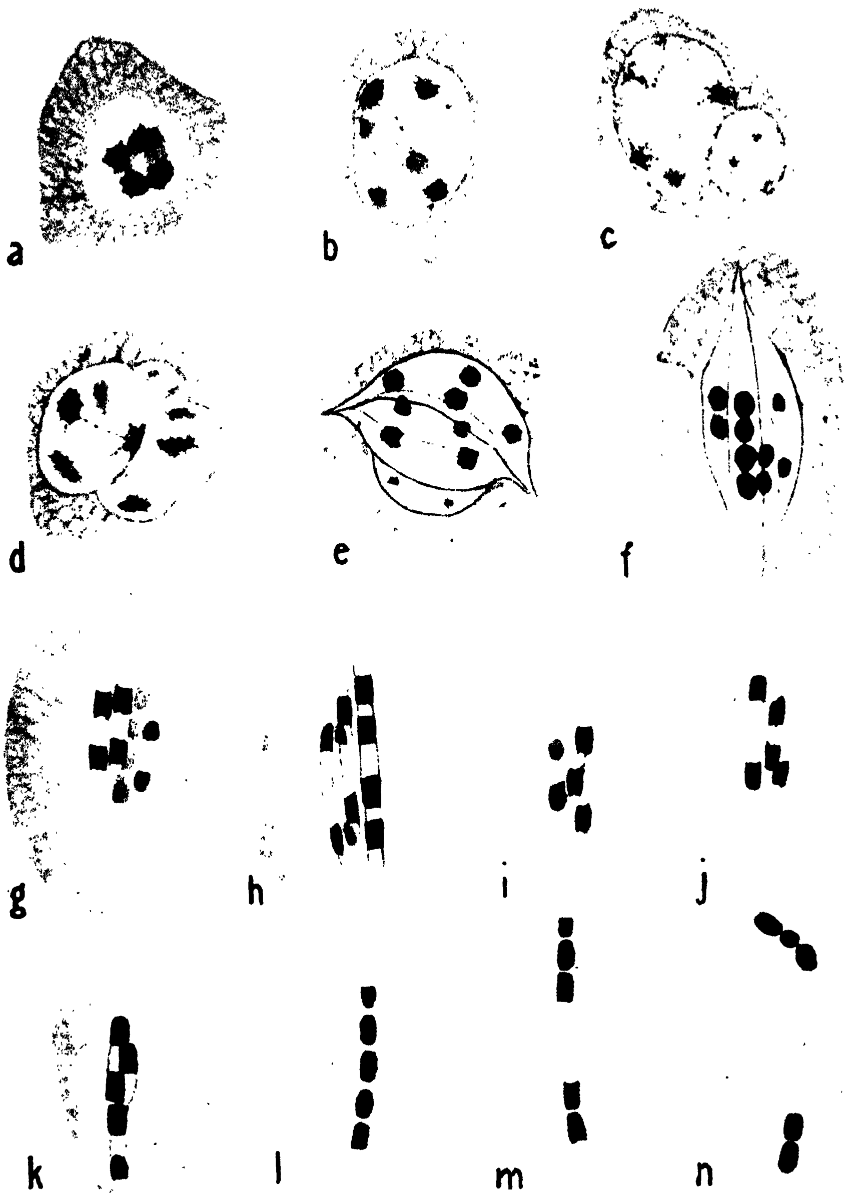


Fig. 20

Protortonia primitiva. *a* – *d*, prophase in first spermatocyte; dissociation of chromatids and vesiculation of nucleus; *e*, *f*, bipolarity established; spindle units forming; *g*, *h*, anaphase I; *i*, *j*, *k*, fusion of precocious anaphase II spindle units during interkinesis; *l*, resultant linear aggregate spindle; *m*, *n*, second division, with X terminal in upper group in *m*, central in *n*. From Schrader (1931).

The vesicles next become elongated along a common axis, closely appressed together, and at either end their attenuated tips converge in a common point (fig. 20, *e*). Within each vesicle the chromatids then reassociate, forming a chain of two in the long axis of each medium-sized vesicle and a chain of four in the large one. The X chromatids orient similarly but without coming into contact with each other (fig. 20, *f*). From the poleward face of each chromatid delicate chromosomal fibers of the divergent, llaveiine type develop during — or even before — the disappearance of the vesicle walls. Anaphasic disjunction then starts, without prior orientation in an equatorial plate, at slightly different times in the different chains of spindle units. Interzonal connectives form between the separating chromatids, always centrally in the chain of four (fig. 20, *g*, *h*), and 5 chromatids accordingly pass into each telophase nucleus.

During interkinesis the chromatids reassociate in pairs whose members are connected by delicate fibers suggesting the interzonals of the first division; the X usually becomes attached to one of the resulting chains (fig. 30, *i*, *j*, *k*). The chains of 2 and 3 chromatids, always parallelly oriented, then become appressed together in such a way that the chromatids of one fit into the interstices of the other and there results a single linear aggregate of all 5 chromatids stretching through the entire length of the cell (fig. 20, 1). The X is terminal, subterminal, or central in position in 74 per cent, 23 per cent, and 2 per cent respectively of 358 cells checked. Divergent chromosomal fibers form at either end of the chain; second anaphase is initiated with the development of a tubular, typically llaveiine interzonal which separates the chain into one group of 3, always including the X, and one of 2 chromatids (fig. 20, *m*, *n*). The further course of spermiogenesis parallels that of Llaveia.

The meiotic mechanism of Protortonia, apparently so anomalous, can be homologized with the basic llaveiine type (Hughes-Schrader, 1931, 1940). Even its most bizarre feature, the curious linear alignment of all the chromosomes prior to the second division is best interpreted as an extreme manifestation of the process already familiar in Llaveiella. The short chains of 2 or 3 chromosomes formed by the realignment of the interkinetic elements are individual spindle units, in one of which the X chromosome becomes trapped. In these spindle units anaphasic disjunction is precocious and asynchronous. The chain of 5 is formed by the fusion of the two precociously and differentially disjoining anaphasic spindle units (fig. 20, *i*–*l*). This fusion apparently establishes a new equilibrium marked by a definite pause — to judge from the relative frequency of the stage shown in Figure 20, 1—after which anaphasic disjunction is resumed but now with a single, joint interzonal connective (fig. 20, *m*). Normal segregation is thus brought about by the same mechanism as in other coccids — the secondary pairing

of chromatids in interkinesis. The non-random position of the X in the chain of 5 results from the fact that it is usually caught up in one of the developing cones of chromosomal fibers (just as in the other llaveiines), and hence is terminal or subterminal; in the small percentage of cases in which its position is central it must have been caught in the interzonal connective of one or the other chain, possible during their fusion.

In the first division the Protortonia story is complicated by the preliminary pairing of the disjoined chromatids. Thereafter the spindle units formed in each of the medium-sized vesicles are typically llaveiine, and their subsequent behavior parallels precisely that of asynaptic chromosomes in the other species. The X chromatids, after their "distance pairing" also give rise to a typical spindle unit, thus presenting the anomaly of an interzonal connective formed without prior contact between the disjoining elements (compare monopolar spindle of lecanoïds, page 179).

But the major problem of the first division is posed by the behavior of the autosome pair included in the single large vesicle. The transient two by two association of its four chromatids prior to their linear alignment must represent the reassociation of sister chromatids preparatory to the first division, which is almost certainly equational for these as for the other chromosomes. To result in an equational division one pair of sister chromatids must occupy a central, while the other two take the terminal positions in the chain of four, — or the members of the two pairs must alternate in the chain. Precisely these orientations would result from the fusion of asynchronous, precociously disjoining, anaphasic spindle units — just as is actually observed in a small proportion of Nautococcus bivalents (compare the linear aggregate BB′ in Nautococcus, top row right, and Protortonia, bottom row, right, fig. 21). Such an origin for the chain of 4 chromatids is supported by the observable asynchrony in anaphase movement of the other, typical spindle units in the division figure. Against it is the fact that actual spindle fibers often do not become visible until after the linear arrangement is assumed. This consideration is perhaps negated by the fact that bipolarity is already established and the vesicles are undergoing a pronounced elongation and a concomitant decrease in diameter. Given a precocity in the initial autonomous disjunction of chromatids, prior to spindle fiber organization, there may well result sufficient separation to allow for the linear interdigitation of the two anaphasic units under pressure from the elongation of the vesicle.

The basic unity of design in the meiotic spindles of Protortonia and the other llaveiines is apparent from the foregoing analysis. In its finished form it is similarly composed of individual chromosomal spindle units, with tubular interzonals, divergent chromosomal fibers, and broadly dispersed poles. Unique among the llaveiines is the transient initial stage

with sharply focussed polar centers formed by the bipolar deformation of the vesicles. This stage is comparable with the meiotic spindle of the iceryines which develops intranuclearly within a similarly bipolarized mem-

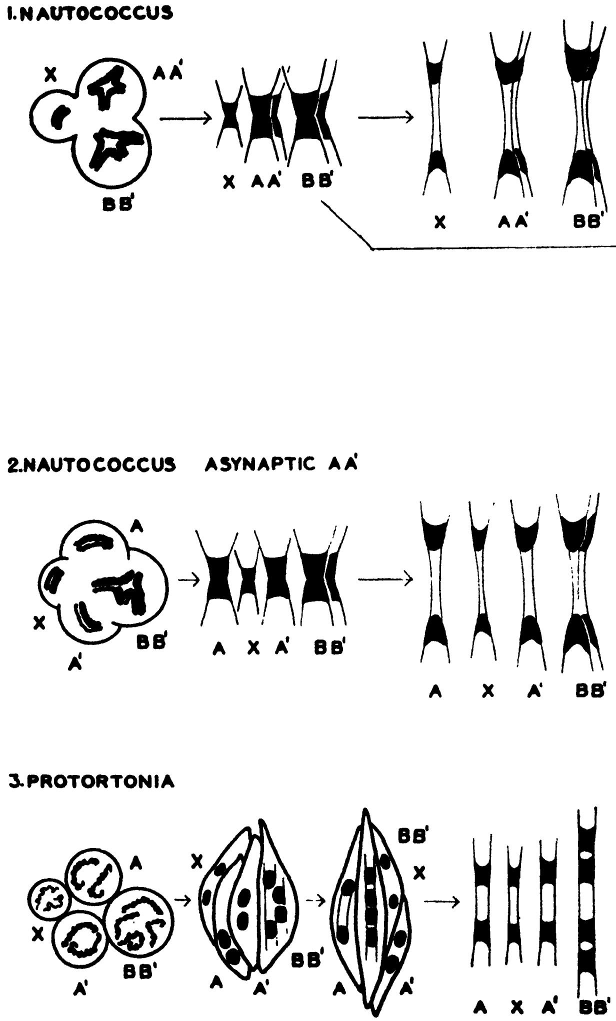


FIG. 21

Diagram — homologies in llaveiine meiotic spindles. 1. Nautococcus; upper row formation of typical spindle units, anaphasic disjunction synchronous; lower row, right, formation of linear aggregate spindle unit by fusion of asynchronous anaphasic units in bivalent BB′. 2. Formation of independent spindle units by asynaptic chromosomes A,A′ in Nautococcus; compare with A,A′ in 3. 3. Protortonia — formation of linear aggregate unit by asynaptic homologues B,B′ through fusion of precocious, asynchronous chromosomal spindle units; comparable with lower row, right, of 1.

brane, — in this case entire rather than vesiculated. The shift from a sharply focussed to broadly dispersed pole — characteristic of meiotic as compared with somatic spindles in all male llaveiines — in Protortonia actually transpires in the one and the same division figure.

The vesiculation of the prophase nucleus, clearly the same phenomenon in Protortonia as in the other llaveiines, bears a significant relation to asynapsis. The formation of a single vesicle around each pair of autosomes, typical of the more generalized species, implies a previous movement of homologous chromosomes to restricted regions of the nucleus — a preliminary pairing. One pair of the Protortonia autosomes, although asynaptic, still performs this first pairing movement; in the other pair even this is lost and a separate vesicle forms about each chromosome precisely as in asynaptic chromosomes of the other species (fig. 21). Additionally in Llaveia, an occasional autosomal vesicle is found with chromosomes spatially separate when first analyzable in diakinesis; these may represent asynaptics of the same type as those of the joint-vesicle in Protortonia. Asynapsis, now fully established for all the chromosomes of Protortonia, may thus have been evolved by different and possibly successive changes in the two bivalents, or by a differential expression of the same mutation by the different autosomes. In all the other llaveiines it is the shorter of the two pairs of autosomes which develops asynaptically and in separate vesicles in a small but constant proportion of the spermatocytes. Unfortunately the size difference between the autosomes has been lost in Protortonia so that it is impossible to say whether, as seems likely, it is the homologue of the larger, more conservative pair that has retained preliminary pairing and a common prophase vesicle. But whatever the steps in its evolution, and whether complete as in Protortonia or partial in expression as in the more primitive species, asynapsis in the llaveiines is always compatible with regular segregation and the production of normal gametes. This is brought about, as pointed out above, by the secondary pairing during interkinesis of homologous chromatids derived from asynaptic chromosomes in the first division. This secondary pairing, as also the equational nature of the preceding division, is not a new modification correlated with asynapsis, but a part of the general coccid heritage. Its persistence has permitted the evolution of asynapsis without the usual sequelae of meiotic irregularities.

C. *The Lecanoïd Type*

With the single exception of Puto (*cf.* p. 134), all species of lecanoïds thus far studied possess a unique type of male meiosis characterized by (1) segregation without prior synapsis or secondary pairing, (2) differentiation in the two haploid sets of chromosomes of distinctive behavior and

coiling cycles, and (3) the suppression of sperm formation in half the gametic nuclei. First discovered by Schrader (1921, 1923c) in several species of Pseudococcus, and later in Gossyparia (Schrader, 1929a), this type is also found in *Phenacoccus acericola* (Hughes-Schrader, 1935) and *P. gossypii* (Hughes-Schrader unpub.) of the same family (Eriococcidae); in the Lecaniidae similar conditions exist in two species of Lecanium (Thomsen, 1927) although technical difficulties here preclude precise analysis; and finally, unpublished observations (Hughes-Schrader) on the cochineal insect Dactylopius sp. (family Dactylopiidae) disclose an identical spermiogenesis. The term lecanoïd for this type of meiosis may well prove too inclusive, since only 3, and these closely related, families out of the 12 now loosely grouped in the section Lecanoïdae have as yet been sampled cytologically.

Phenacoccus acericola (Hughes-Schrader, 1935) may be taken as a type. The diploid number in both sexes is 12, with no differentiation of sex chromosomes. Somatic and meiotic mitosis in the female are orthodox. In the male, however, one haploid set of chromosomes maintains, from the blastula stage on, a permanently contracted state, fusing into a heteropycnotic mass during resting phases and separating into its constituent chromosomes for each mitosis.

In meiotic prophase the 6 chromosomes comprising the heteropycnotic mass separate and assume a plate-like orientation (fig. 22, *a*, *b*), after which the 6 predominantly euchromatic chromosomes condense as typical univalents (fig. 22, *c*). By first metaphase the chromosomes of the two sets are equally contracted and indistinguishable. All 12 chromosomes divide equationally at the first division (fig. 22, *d*, *e*). In each anaphase group 6 chromosomes, presumably those derived from the heteropycnotic set, remain condensed and tend to clump while the other 6 show no aggregation and become progressively less compact (fig. 22, *f*, *g*). From the heteropycnotic chromosomes typical chromosomal fibers develop and, converging in a centriole, form a half or monopolar spindle (fig. 22, *h*). In the ensuing division these fibers shorten and the heteropycnotic chromosomes move toward the single pole, while delicate interzonal fibers appear between the two chromosome sets (fig. 22, *i*, *j*). The heteropycnotic chromosomes form a small, increasingly pycnotic nucleus. Cytoplasmic cleavage is suppressed and subsequent fusion of sister cells produces the quadrinucleate spermatids characteristic of coccids (fig. 22, *k*, *l*). In each spermatid the nuclei derived from the euchromatic chromosomes form normal sperm; the pycnotic nuclei degenerate in situ (fig. 22, *m*, *p*). The cytological demonstration of this process rests not only on closely seriated stages but is confirmed by the fact that the number of nuclei per cyst at second telophase is precisely twice the number of sperms formed from each cyst.

Pseudococcus (Schrader, 1921, 1923c) conforms in essentials to the Phenacoccus type. Schrader's original report that both types of spermatid nuclei give rise to sperm appears erroneous in view of the close correspond-

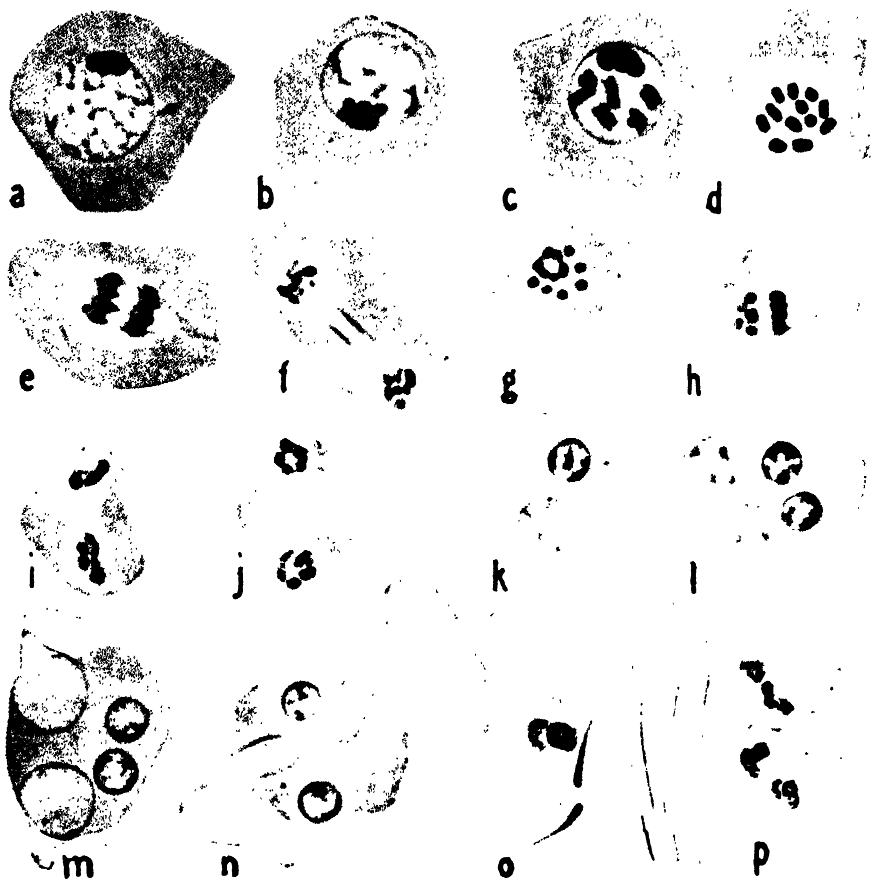


FIG. 22

Meiosis in male *Phenacoccus acericola*. *a*, *b*, early prophase I with separation of heteropycnotic mass into 6 univalents; *c*, late prophase I — contraction of 6 euchromatic univalents; *d*, metaphase I; *e*, *f*, anaphase I; *g*, metaphase II; *h*, monopolar spindle from heteropycnotic set; *i*, *j*, anaphase II; *k*, spermatid; *l*, fusion of sister binucleate spermatids; *m*, *n*, *o*, degeneration of heteropycnotic nuclei; formation of sperm from euchromatic nuclei; *p*, discarding of pycnotic nuclei with cell body; mature sperm at left. From Hughes-Schrader (1935).

ence in spermiogenesis obtaining between Pseudococcus and the other lecanoïds, Phenacoccus, Gossyparia, and Dactylopius — in all of which the degeneration and loss of those nuclei derived from the heteropycnotic

set can be established beyond question. Significant variations from the Phenacoccus type relative to heteropycnosis and spindle formation, however, are definitely established. The three species investigated, *Pseudococcus nipae*, *P. maritimus*, and *P. citri*, show different degrees of heteropycnosis, decreasing in the order named and in none so extreme as in Phenacoccus. Preparatory to meiotic prophase considerable despiralization of the heteropycnotic chromosomes occurs, so that they are represented by a cluster of chromocenters instead of a single heteropycnotic mass. In the ensuing prophase they give rise to normal appearing but precociously contracting chromosome threads, each with a clear equational split and a tendency, especially in *P. citri*, to assume a transient bouquet orientation (fig. 23, *a*,

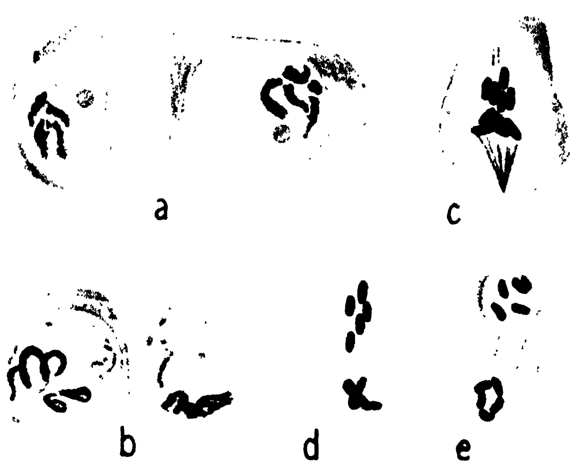


Fig. 23

Selected stages in male meiosis, Pseudococcus. *a*, *c*, *d*, *e*, *P. maritimus*, *b*, *P. citri*. *a*, prophase I, heteropycnosis slight; *b*, heteropycnotic chromosomes in bouquet orientation; *c*, metaphase II, transient chromosomal fibers from euchromatic set; *d*, *e*, division II. From Schrader (1923c).

b). A less extreme difference in the coiling cycles of the two haploid sets is again evident in the second division (fig. 23, *c*, *d*, *e*); here the chromosomes of the two sets appear equally contracted and the heteropycnotic tendency of one set is apparent only in the close aggregation of its members. Associated with the foregoing is the formation of a few irregular chromosomal fibers by the non-heteropycnotic chromosomes. The latter, however, remain passive in the actual division, and segregation on the monopolar spindle organized by the heteropycnotic chromosomes occurs just as in Phenacoccus. Such interspecific differences in degree of heteropycnosis, taken in conjunction with the persistence of the primitive coccid meiosis

in Puto, a genus of the same family, justifies hope that further comparative studies within the group may yield evidence on the evolution of the specialized lecanoïd meiotic mechanisms.

Gossyparia spuria (Schrader, 1929a) presents extremely puzzling complications of the Phenacoccus scheme. The diploid number is 28 in both sexes. The tendency to permanent heteropyenosis of one haploid set is apparent in the male from late blastula on. As in Pseudococcus, this is less marked in early meiotic prophase than in somatic mitosis, indeed in Gossyparia even the collocation of chromocenters disappears. But the outstanding feature of the Gossyparia meiosis lies in a pairing of the 14 heteropycnotic chromosomes during this diffuse stage. From the latter they condense, precociously and asynchronously, as 7 compact chromosomal elements which aggregate, as they form, in a plate arrangement (fig. 24, *a–c*). The 14 more euchromatic chromosomes then condense as

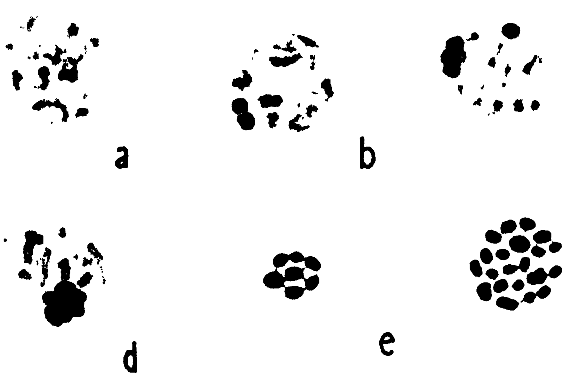


FIG. 24

Selected stages in male meiosis, *Gossyparia spuria a*, early prophase I, no heteropycnosis; *b–e*, asynchronous condensation of 7 heteropycnotic "bivalents"; *f*, metaphase I with 7 "bivalents" and 14 univalents. From Schrader (1929a).

typical univalents, and the first metaphase plate thus regularly carries 21 chromosomes of which 7 are bivalent (fig. 24, *f*). Occasional plates with 22 or 23 chromosomal bodies indicate sporadic secondary disjunction of one or two of the bivalents. All chromosomes divide at the first division. Thereafter the 7 dyads derived from the heteropycnotic set remain contracted and clumped, give rise to a monopolar spindle, and segregate from the looser group of 14 univalents. The latter are completely passive in the second division and despiralize progressively to form a typical spermatid nucleus. The heteropycnotic chromosomes degenerate as in the other species. In one male, of 30 studied, initial spermiogenetic changes were observed in

the pycnotic nuclei, but these were obviously abnormal and blocked at an early stage.

Clearly the basic departure of Gossyparia and one that should give a clue to its origin lies in the peculiar pairing of the heteropycnotic chromosomes. White (1945, 1946b) has suggested that Gossyparia is tetraploid and that the doubling of the chromosome number has restored pairing among the heteropycnotic chromosomes. Were this true a segregation to opposite poles of the chromatids of each heteropycnotic dyad should be expected in the second division. Alternatively we may assume in Gossyparia a permanent separation of chromatids such as is occasionally observed in the X chromosome of Llaveiella and occurs regularly in all meiotic chromosomes of Protortonia. In all cells of the female Gossyparia and in male soma the chromatids might then behave consistently as chromosomes, but reassociate again at meiotic prophase, just as they do in Protortonia, prior to their equational separation in the first division. If the pairing be of this nature no disjunction of the heteropycnotic dyads is to be expected at the second division, rather, they should segregate, as they do, in a group from the other haploid set. No conclusion is warranted without further evidence which may well be forthcoming when other eriococcines, and especially other species of Gossyparia, are investigated. This is the more likely since the American *G. spuria* analyzed by Schrader is a naturalized import from Europe; the other species now known are exclusively Australian and European. Diploid chromosome numbers of 10, 12 and 14 characterize the 6 species of Eriococcidae now known cytologically; this is at least compatible with the idea that a doubling of chromosome number has occurred in the history of *G. spuria*.

Turning now to more general considerations, it is clear that cytologically two fundamental developments underlie the evolution of lecanoïd male meiosis — asynapsis, and differential coiling cycles in the chromosomes of the two haploid sets. Asynapsis we have seen to be a frequently recurrent development in coccids. In lecanoïds, unlike llaveiines, the change has involved the suppression both of synapsis and of secondary pairing, — agreeing in this respect with the modification of female meiosis in obligatorily parthenogenetic females of Lecanium. Differential coiling cycles among the chromosomes of a complement are also well established among coccids. They are found in spermiogenesis of iceryines, and may be a factor in the anaphasic asynchrony so prevalent in llaveiines. Peculiar to Gossyparia is the variation among members of one haploid set only, seen in the asynchronous contraction of heteropycnotic bivalents. Characteristic of the higher lecanoïds as a group is the development by whole haploid sets of differential cycles. The tendency for one haploid set to maintain the tightly coiled, contracted state, even to the extent of a

permanent heteropycnosis, is associated with a precocity of this set in mitosis. To such precocity might be ascribed the correlated development of asynapsis in lecanoïds, since synapsis presupposes a similar state of extension in the pairing homologues. Schrader (1929a) has further suggested that the atypical nature of the second division, with its monopolar spindle and consequent segregation without prior pairing, results directly from the differential coiling cycles of the two haploid sets. Precociously condensed, the heteropycnotic chromosomes reach the metaphase stage of contraction and induce spindle formation before the euchromatic set is ready to participate. In this conception the coiling cycle of the latter is considered normal but so delayed relative to that of the heteropycnotic set as never to reach second metaphase. Some support for this interpretation lies in the incipient formation of non-functional chromosomal fibers by the euchromatic chromosomes of Pseudococcus which would thus be attributable to the slighter differentiation of the two sets distinguishing that genus from the others. Opposed is the fact that the same precocity of heterochromosomes has no such effect on the division figure in the first meiotic and somatic mitoses; here anaphasic movement is delayed until the slow group has completed contraction and orientation, and a normal bipolar spindle results. Furthermore, there is in both Phenacoccus and Gossyparia some evidence that the coiling cycle of the euchromatic chromosomes has been modified in more respects than its relative timing. In these two species, from first meiotic telophase on, the euchromatic chromosomes undergo a progressive despiralization culminating in the resting phase of the spermatid nucleus. All chromosomal activity associated with a second division — metaphase orientation, spindle fiber formation, and anaphasic movement — are suppressed; the meiotic behavior of this set of chromosomes parallels that in the haploid male of iceryines and the obligatorily parthenogenetic eggs of Lecanium. If Gossyparia prove to be tetraploid, the failure of its euchromatic chromosomes to undergo secondary pairing, although present in the diploid number and in similar degree of extension, will afford further evidence that the suppression of the second division in this set is genetically determined and not simply a mechanical result of asynchrony. Admittedly such a conclusion only complicates the problem of how such differences in chromosome behavior are transmitted. They are not determined by the individual chromosome since heteropycnosis in a given male involves chromosomes which were euchromatic in the preceding generation. It is possible, as White (1945) has suggested, that prior conditioning in male environment of one set of chromosomes induces heteropycnosis in that set when again brought into a male. An additional assumption is, however, necessitated: such conditioning of the paternally derived set must also induce in the maternally derived chromosomes the

correlated suppression of second division activity. The significance of the heteropycnosis is further considered, in connection with the problem of sex determination.

VI. The Mitotic Division Figure

Great plasticity of the mitotic mechanism is evident in the evolution of the chromosome cycles just reviewed. Underlying the resultant diversity in division figure a unity of plan and homology of parts has been demonstrated. The diagnostic features of the basic coccid mitosis are those of Homoptera and Heteroptera in general; they stem from the diffuse nature of the kinetochore and involve, as already described, parallel anaphasic disjunction, and characteristic metaphase orientation and chromosomal fiber formation. With this common denominator have evolved division figures with spindles of intranuclear, linear aggregate and monopolar types, and bipolar spindles with dispersed poles and divergent chromosomal fibers. In comparing these types certain factors are recognizable which are significant alike for the mechanics of mitosis and the evolution of the coccid division figure.

First, as regards the cell organelles or structural components involved in mitosis, coccid figures present a simplification of the usual apparatus. Mitosis is possible with no new structural differentiations in the cell other than chromosomal fibers and interzonal connectives. Asters and centrioles may be absent, and in none of the variant coccid figures are any continuous spindle fibers demonstrable. The spindle thus appears to be compounded solely of individual chromosomal spindle units. Each unit comprises at metaphase one chromosome and its two masses of chromosomal fibers, and at anaphase two daughter chromosomes with chromosomal fibers and interzonal connectives. In those llaveiines in which chromosomal fibers form prior to the establishment of the bipolar axis, this compound structure is especially clear, for here the spindle actually arises through the gradual collocation of previously formed chromosomal spindle units.

Absence of continuous fibers does not, however, preclude the existence of a spindle body, organized from the nucleoplasm, distinct from the chromosomal spindle units, and comparable except for the lack of fibrillar differentiation with the central spindle of other organisms. Some support for this suggestion lies in the frequent persistent demarcation of nucleoplasm from cytoplasm after the dissolution of the nuclear membrane, as pointed out by Ris, (manuscript in preparation). Furthermore, in the Steatococcus spermatocyte the intact nuclear membrane assumes the shape of a spindle and into its opposed and extended tips the chromosomal fibers later converge. This may well reflect the organization of the nucleoplasm into a central spindle body. Of similar nature may be the molding

of the prophase vesicles of the Protortonia spermatocyte into a compound, karyomeric spindle-shaped configuration prior to the formation of chromosomal fibers. Opposed to the conception of such a spindle body is the observation, in living spermatocytes of Llaveiella, that on rupture of the cell wall the individual chromosomal spindle units may be moved freely about by currents in the medium; they do not appear to be held together by a central spindle body (Hughes-Schrader, 1942). Ris sees no contradiction here, since he considers each spindle unit of the Llaveiella type of meiotic figure to be a separate karyomeric spindle. But even if we accept this interpretation, it must be admitted that no spindle substance other than interzonals and chromosomal fibers is here demonstrable during the late anaphasic elongation.

The possible existence of a non-fibrous central spindle body bears directly on the question of the anaphasic movement of chromosomes in coccid mitotic figures. That the chromosomal fibers are involved in the movement of chromosomes to the pole may be assumed from their progressive shortening during anaphase. That this is not the only factor is evident in the increased distance between the poles at telophase compared to metaphase (figs. 15, 16). To ascribe this elongation to increase in length of the interzonal connectives is a natural conclusion in the case of the Llaveia and iceryine spermatocytes. Here the stembody, formed by a fusion of the interzonals, actually increases in length until the telophase nuclei are pushed aside against the cell wall and may even continue to elongate until its ends protrude as definite projections on the periphery of the cell (fig. 16, *c*, *h*). In the later stages of this process the cytoplasm and its contained granules impinge directly upon the stembody — no nucleoplasm extraneous to the interzonals is any longer discernible — and it would therefore appear that the elongation of the stembody in its latter phase at least is independent of any surrounding spindle body. But such stembodies are by no means common to all the variant types of coccid mitotic figures; nor can elongation of the stembody and shortening of chromosomal fibers alone explain the total configuration even in the case of the llaveiine meiotic figure. Already in 1931, Schrader in his analysis of the Protortonia figure recognized the existence of two factors determining the metaphase and anaphase configurations — one intra-chromosomal, functioning through chromosomal fibers and interzonals, and one extra-chromosomal, expressed in the elongation of the vesicles and the transverse pressure on the spindle units. The action of the extra-chromosomal factor is apparent in the meiotic figure of other llaveiines also (Hughes-Schrader, 1940), in bringing together the anaphasic spindle units and facilitating the formation of linear aggregates of chromosomes. Is Schrader's extra-chromosomal factor to be identified with changes in shape of a non-fibrous

spindle body? Ris (1943, and manuscript cited above) has shown, through measurements on living and fixed cells, that anaphasic movement of chromosomes depends on two separable factors, elongation of the central spindle, and shortening of the chromosomal fibers. Ris further attempts to explain the coccid division figures in terms of these same forces, citing in support the monopolar spindle of lecanoïd spermatocytes. Here, while the heteropycnotic chromosomes are apparently moved to the single differentiated pole by a shortening of their chromosomal fibers, the distance separating the two sets of chromosomes becomes much greater than the original length of the chromosomal fibers (figs. 22, 23). Elongation of a spindle body, here represented by nucleoplasm still visibly demarked from the cytoplasm, is almost certainly indicated. Furthermore there exists a striking similarity between certain mitotic figures of coccids and of the closely allied aphids in which Ris has demonstrated the action of both spindle body and chromosomal fibers. In the aphid Ris finds no evidence of the functioning of a stembody, nor is one structurally differentiated; coccid figures vary from the usual somatic type in which interzonals are delicate and transient and no stembody is demonstrable to the llaveiine meiotic type in which strongly differentiated interzonals, formed in close association with the chromosomes, fuse into a structurally distinct stembody. For the present it seems most reasonable to assume that in coccids a spindle body devoid of continuous fibers may be variably developed in different mitotic types and that its function in anaphasic disjunction may in certain forms be supplemented by the elongation of a stembody of interzonal origin.

The degree of concentration of the poles or division centers is known to vary widely both within a given chromosome cycle and among different organisms. In coccids a wide range of conditions is realized, from concentrated poles with chromosomal fibers converging to a focal point which may or may not be marked by centriole or aster, to broadly dispersed poles or polar regions unmarked by any structural differentiation and with distally divergent chromosomal fibers. The Protortonia spermatocyte shows additionally that within a single mitotic configuration the pole may pass from a concentrated to a broadly dispersed state.

The relative timing of the establishment of bipolarity in the cell and the formation of chromosomal fibers is also shown to be capable of variation which has played a part in the evolution of the division figure in coccids. Chromosomal fibers may form prior to the establishment of bipolarity, as in most llaveiine spermatocytes, or subsequent thereto, as in Protortonia and the majority of other forms. The former cases demonstrate that the formation of chromosomal fibers is initiated at the kinetochore. One must further conclude either that the organization of these fibers may proceed

from the kinetochore alone without interaction with a division center, or, that the pole usually represented by a more or less concentrated center is peripherally dispersed. Certainly the chromosomal fibers when first formed are oriented only with reference to the individual chromosome concerned.

The relative persistence of the nuclear membrane constitutes another variable in coccid mitosis. In most types it disappears in late prophase; in the Protortonia spermatocyte, represented by the walls of the vesicles, it may persist through the initial phases of the precocious anaphase; and in spermatocytes of many iceryines it remains intact throughout the entire anaphase. In the last named cases there can be no question of the completely intranuclear origin of the spindle; and even in the other types nuclear origin of spindle components is almost certain. Only in those figures in which centrioles and asters occur can any structural components of the spindle be certainly attributed to the cytoplasm. The localization of the pole determinants in the acentric spindle types is unknown, but may well be cytoplasmic there also.

Autonomy of the chromosomes and of its constituent units, expressed in asynchronous formation of chromosomal fibers and asynchronous incidence and rate of anaphasic disjunction in meiosis of male llaveiines, is of significance both for the mechanics of mitosis and the evolution of the coccid division figure. The data stand in contradiction to any hypothesis of anaphasic movement based on currents, repulsions, or other forces acting simultaneously and similarly on all chromosomes alike. Conversely the attribution to the spindle unit with its individual chromosomal fibers and interzonals of a role in anaphasic movement allows for differential behavior among chromosome elements and is in harmony with the data on asynchronous chromosomes. In the evolution of coccid figures, the unique type of spindle or spindle unit in which multiple chromosome elements are aligned in linear sequence in the interpolar axis also stems in part from asynchrony, since it results from fusion of asynchronous anaphasic spindle units. Such linear aggregates may comprise disjoining chromatids, half chromatids, or even quarter chromatids, since the constituent elements of a chromosome form separate spindle units depending on the degree of their spatial separation. Linear aggregates occur with low and variable frequency in the first meiotic division of the Nautococcus spermatocyte, and with higher frequency in the second division of the Llaveiella. In Protortonia the process has become completely stabilized as a regular part of male meiosis. In the first division the four chromatids of one pair of autosomes invariably form a linear aggregate within a single spindle unit. In the second division the fusion of the precocious and asynchronous anaphasic spindle units of both autosome pairs gives rise to the most

bizarre of all coccid figures, in which all the chromosome elements of the cell are linearly aligned within a single spindle unit.

An extreme plasticity in the metaphase and anaphase chromosome contributes to the variation in coccid division figures. This is most striking in the deformation of chromosomes in the elongating anaphasic spindle units of llaveiine spermatocytes (figs. 16, 18, 19). At each stage the shape of the chromatid or half chromatid conforms precisely to the changing outlines of the spindle unit and its distal edges are extended in a delicate flange along the boundary between cytoplasm and chromosomal fibers. This deformation is almost certainly referable to redisposition or distortion of matrical substances and does not necessarily affect the orientation of the chromonemata (compare the spectacular deformation of the X chromosome in the aphid Tamalia analysed by Ris, 1942). The plasticity indicated by these changes of shape appears to be a general character of heteropteran and homopteran chromosomes and should be taken into account in the analysis of chromosome orientation and inter-relations in this group.

Finally, mention should be made of the effect on the division figure of the mitotic inactivity of half of the chromosomes, such as occurs in the second division of lecanoïd spermatocytes. That a monopolar spindle results is correlated with the fact that this is, however atypical, still a reduction division; the mitotically active chromosomes accordingly segregate from their inactivated homologues rather than divide. This poses the identical problem presented by the orientation and segregation of many unpaired sex chromosomes. What special mechanism underlies the orientation of such chromosomes in a metaphase-like plate and the formation and functioning of a single half spindle of chromosomal fibers is unknown; singleness or doubleness of the kinetochore is excluded, since the paired chromosomes of the heteropycnotic set in Gossyparia also segregate without separation precisely as do the univalents in the other lecanoïds. After orientation the anaphase movement involves, as already noted, shortening of chromosomal fibers and elongation of the figure as a whole, justifying Ris's term, a "monocentric bipolar spindle" for the second meiotic division figure in lecanoïds. Caution in the extension of this principle to all monopolar spindles is, however, advisable in view of the conditions presented by the beetle Micromalthus (Scott, 1938).

VII. Sex Determination

A. *XO — XX Sex Chromosome Mechanism*

An XO-male, XX-female sex chromosome mechanism may safely be regarded as a part of the primitive heritage of the coccids, since it is found

in all other Sternorhynca investigated and has persisted unchanged in the more primitive coccids, such as the Llaveiini — a tribe of the most primitive family of the generalized section Margaroïdae — and in Puto, which on both cytological and general taxonomic criteria represents a persistent primitive, linking the margaroïd and lecanoïd stems. The meiotic behavior of the sex chromosomes has already been reviewed under the groups involved. From a more comprehensive point of view it is of interest that, while in Puto and in Matsucoccus (to be described later), the X passes undivided to one pole in the first and divides in the second meiotic mitosis, the reverse order is followed in all the Llaveiini. Segregation in the first division is probably primitive for coccids since it is the rule in other Homoptera and indeed in most organisms with XO or XY males. Similar shifts in the sequence of segregation and division of sex chromosomes in meiosis have occurred in the Heteroptera, Odonata, and certain Coleoptera (discussion in White, 1945).

Structural modifications of the X are apparent in the Llaveiini. In two species of Llaveia, *Llaveiella taenechina*, and *Nautococcus schraderae* corresponding autosomes are remarkably uniform in size while the X is the largest of the set in Llaveia and the smallest in the other two species. While Protortonia has the same number of chromosomes, these deviate in both absolute and relative size from those of other llaveiines, and its X, again the smallest of the complement, is negatively heterochromatic in male meiotic prophase. Within the Llaveia, Llaveiella, Nautococcus section, the derivation of the short X of Llaveiella by fragmentation and loss of fragments which in Nautococcus have persisted as supernumeraries is quite possible although not demonstrable cytologically.

B. *Compound Sex Chromosomes*

A compound sex chromosome mechanism of the type familiar in several heteropteran families, in which the X is represented by 6 chromosomes instead of one, is found in *Matsucoccus gallicola*, — the only species thus far investigated of the subfamily Xylococcinae of the Margarodidae (unpublished data, Hughes-Schrader). The diploid number of the male is 34, of the female 40. In male meiosis the 6 univalent X chromosomes condense in advance of the others, and orient at first metaphase in a ring or plate surrounded by the 14 more slowly condensing bivalents. The compound X chromosomes move as a unit to one pole in the first division. Secondary spermatocytes are thus of two types, with 14 and 20 chromosomes respectively, and the second division is equational for the X's. The discovery of a compound sex chromosome mechanism in coccids is not surprising since the chance of its development is greatly augmented by the diffuse nature of the kinetochore which should permit its evolution by simple

fragmentation of an originally single X (Schrader 1947.) Significant in this connection is Ris' (1942) demonstration in one of the aphids (a group closely related to coccids) of some mitotic activity by the fragments of an experimentally broken X chromosome. Origin through simple fragmentation in the case of Matsucoccus is supported by the fact that the 6 X chromosomes are heterochromatic throughout their length in male meiotic prophase; there is no evidence of recent translocation of euchromatic sections of autosomal origin. Further evidence may lie in the meiotic behavior of the 12 X's of the female, but these data are not yet available (compare Troedsson's analysis in reduviid females, 1944).

C. *Sex Determination in the Lecanoïdae*

Briefly stated the problem of sex determination in the specialized lecanoïds is — how are two sexes produced from eggs cytologically identical and fertilized by sperm derived from only one of the two haploid chromosome sets of the male? The problem is insoluble on cytological data alone and must await genetic analysis. Certain implications of the data now available should, however, be considered.

In the first place it is clear from the evidence just reviewed that the lecanoïd condition has evolved from the XO — XX mechanism through the loss of the sex chromosomes. That heterogamety of the male was sacrificed in the evolution of an alternative sex-determining mechanism is equally clear since only one haploid set of chromosomes now produces sperm. It would be valuable to know whether the haploid set which degenerates in spermiogenesis is of maternal or paternal derivation or comprises a mixture of the two. Direct observation is impossible since the heteropycnosis of the male does not develop until a late blastula stage; indirect evidence may lie in Dickson's (1941) work on the inheritance of resistance to hydrogen cyanide fumigation in the California red scale, *Aonidiella aurantia*. Survival value in F_2 from either parental combination of resistant and susceptible strains lies approximately midway between the value of the respective F_1 and that of the type to which the paternal grandmother belonged. Assuming a lecanoïd male meiosis these data indicate the elimination of the paternally derived chromosomes in spermiogenesis. No final conclusion is warranted, however, since the crucial cytological data are lacking for the highly specialized family Diaspididae to which Aonidiella belongs. If future work substantiates the assumption — and it is a big one — of a lecanoïd meiosis, certain further inferences as to the latter may be drawn. We may then attribute to a prior conditioning in the male the development of heteropycnosis by one haploid set of chromosomes in embryos of male constitution. A possible parallel is afforded by Sciara (Crouse, 1946) when an X chromosome "conditioned"

by an immediately preceding passage through a male, induces among other changes heteropycnosis in the meiotic chromosomes. This raises the question of the nature of the heteropycnosis in male lecanoïds. Schrader (1929a) and Schrader and Hughes-Schrader (1926) have interpreted it as inactivation or degeneration inducing a physiological haploidy in the male. If the Aonidiella data prove applicable to lecanoïds we must conclude that both paternal and maternal chromosome sets are active somatically in the male, since the F_1's from reciprocal crosses of resistant and susceptible strains give the same survival value. But admitting the probability of somatic activity of the heteropycnotic set, the fact remains that these chromosomes do degenerate and are eliminated in spermiogenesis; the lecanoïd male therefore does breed as a haploid. Even the euchromatic set of chromosomes takes on the meiotic behavior of a haploid, dividing equationally in the first and remaining passive during the second division.

But although the male breeds as a haploid, sex determination is not of the haplo-diploid type since male and female alike develop from fertilized eggs. Sexual differentiation among the embryos must here depend either on a cytologically invisible digamety of the female, on environmental control, or on a combination of the two. Environmental influence on sex determination is indicated by the data on sex ratio. As was already evident in Schrader's (1923a) breeding experiments and has been thoroughly established in the extensive and careful studies of James (1937, 1938, 1939) the sex ratio in Pseudococcus fluctuates widely from species to species and within the species. In five species studied by James the mean sex ratio of a large series varies from 19 to 101 males per 100 females. Broken down into individual broods, the variation in family sex ratio within each species is as great or greater than that between species. The variation is not correlated with size of brood; high and low values occur alike in broods which approach the potential maximum of the female. Humidity, temperature, and food were carefully controlled. Varying environmental factors, James finds that age of the female affects sex ratio. In *P. citri* the number of males per 100 females increased from 101 at normal mating period of female, to 180 after a 6 weeks delay in mating, 327 after 8 weeks, and 991 after 10 weeks. The total progeny per female is normal after the 6 weeks aging, decreasing thereafter to 54 per cent of normal after 10 weeks delay. Differential mortality is thus excluded as a factor in the 6 weeks experiment. Dryness of the air depresses the sex ratio from overaged females. The mean sex ratio of broods from 35 females overaged 6 weeks in dry air (R.H. 4.76 per cent) was 1.53, in a similar series with relative humidity 4.05 per cent the ratio was 1.64, — in contrast with a ratio of 1.80 from a comparable series overaged in saturated air. The variation is statistically significant; dryness of air favors female production

when superimposed on the male determining influence of delayed mating of the female.

The conclusion seems unavoidable that the mechanism of sex determination is unstable and modifiable by environmental influences. Of the genetic mechanism involved nothing is known. Two factors limiting any working hypothesis proposed are, however, already apparent: (1) no intersexes have been observed in the process of rather extensive breeding experiments with sexual races and species; and (2) no sex types other than normal females are produced by the facultatively parthenogenetic race of Lecanium species in which diploidy is restored by fusion of the egg pronucleus and the second polar body. Obviously, the genetic mechanism underlying sex determination in lecanoïds must await experimental analysis.

D. *Haplo-diploid Sex Determination*

In the Iceryini also the genetic mechanism of sex determination is unknown. Cytologically the system is based on haploidy of the male and diploidy of the female, dependent in turn on whether the eggs, which undergo normal reduction, develop parthenogenetically or are fertilized. The genic balance system of sex determination, assumed from the presence of sex chromosomes in primitive coccids to be the ancestral condition, is not operative here since the ratio of male to female sex determining genes is the same in haploid and diploid. Formal explanation is possible along the lines of hypotheses advanced by Goldschmidt (1920) and Schrader and Sturtevant (1923). The former suggested that precocious activity of male determining genes, in diploid dose prior to reduction, predisposes all eggs to male differentiation which is then overridden in fertilized eggs by the delayed activity of two sets of female determining genes. In the algebraic sum hypothesis of Schrader and Sturtevant also sex differentiation in haplo-diploid animals is harmonized with that of other organisms, sex depending on an additive relation between opposing factors. But neither hypothesis is as yet supported by experimental evidence in haplo-diploid animals.

In only one instance — the parasitic ichneumonoid wasp, *Habrobracon juglandis* — has the actual genetic mechanism of haplo-diploid sex determination been discovered. Here the Whitings and their co-workers have shown sex to depend on the interaction of multiple sex alleles (or differential chromosome segments), heterozygosity for which induces female — homozygosity male — differentiation. The haploid is accordingly male, the heterozygous diploid female. Diploid homozygotes arise from close inbreeding; they are male, but of low viability, and sterile. Whiting (1945) has attempted to extend to all haplo-diploid animals the Habrobracon scheme of sex determination. That so complicated a mechanism should have

evolved independently in the 5 or 6 taxonomically widely disparate groups of haplo-diploid animals seems unlikely on a priori grounds. In the iceryine coccids a natural experiment negating the hypothesis is afforded by the hermaphrodite *Icerya purchasi*. Here the closest possible inbreeding (self-fertilization) fails to produce diploid males — nor has extensive breeding and cytological work yielded any evidence indicative of zygotic, embryonic or nymphal mortality of diploid homozygotes. Even within the Hymenoptera diploid males are unknown in the proctotrypoid wasp *Telenomus fariai* (Dreyfus and Breuer, 1944) which reproduces regularly by brother-sister mating. And again, Smith (1941) has pointed out that in thelyotokous sawflies which restore diploidy by fusion of egg pronucleus and second polar body, sex heterozygosity of females could be maintained only by special regulation of crossing over. Finally, Schmieder and Whiting's (1947) analysis of egg counts and viability ratios in the chalcidoid Melittobia has definitely demonstrated the absence of diploid males and inviable eggs or young ascribable to sexually homozygous zygotes. Since even within the Hymenoptera the genetic sex mechanism operating in closely inbreeding forms is thus different from that of Habrobracon, it is clear that no extension of the principle to other haplo-diploid groups is justified; the mechanism involved must in each case await experimental analysis.

The evolutionary origin of haplo-diploidy has recently been reviewed by White (1945) and Whiting (1945); only the case of Iceryini will be considered here. As in the lecanoïds, its derivation from a system in which both sexes are diploid and sex is dependent on an XO-XX sex chromosome mechanism is clear. It seems plausible that in both lines during the transitional period of elimination and transformation of sex chromosomes, there was built up a reservoir of chromosomal types differing in their content of sex genes out of which the new genetic sex determining mechanisms were evolved. The lecanoïd and iceryine systems although so diverse cytologically have in common the fact that in each the male breeds as a haploid. Complete male haploidy and stabilization in a haplo-diploid system in the iceryines involved additionally the acquisition of viability in the haploid state and the removal of the block to development in reduced, unfertilized eggs. Schrader and Hughes-Schrader (1926) have suggested that attainment of haploidy in the iceryine male involved, in addition to the foregoing, a series of changes in the chromosome cycle of the male. These changes comprised progressive asynapsis, which in turn became correlated with a progressive asynchrony in the functioning of the haploid sets, and culminated in permanent heteropycnosis in, and suppression of sperm formation by, one haploid set of chromosomes. The steps in such a series are actually realized in male meiosis in various llaveiine and lecanoïd species, — and while the species concerned obviously stand in no

such phylogenetic relation to each other, they indicate the type of changes capable of establishment in coccid chromosome cycles — some or all of which may have been instrumental in or incidental to the establishment of haploidy in the Iceryini. Whiting (1945) criticises this interpretation on the basis that asynapsis, heteropycnosis, and male haploidy are unrelated phenomena, citing their mutually independent occurrence in other organisms. But the hypothesis involves no generalization extending to the other groups, and is based on the fact that in lecanoïds the three phenomena are actually intimately associated: asynapsis follows on asynchrony of haploid sets induced by heteropycnosis, and heteropycnosis culminates in suppression of sperm formation from one haploid set with the result that the male breeds as a haploid. To what extent the Schraders' further interpretation of lecanoïd heteropycnosis as inactivation or degeneration inducing a physiological haploidy is warranted only genetic analysis and a determination of the functions of the heterochromatic state will tell.

VIII. Cytology and Taxonomy

The very diversity of cytological mechanisms among coccid groups should eventually make cytological data more than ordinarily useful in the development of a phylogenetically significant taxonomy. Already this is beginning to be realized. The taxonomic position of the cytologically known species is shown in Table 1, p. 130, based on the most recent classification of major groups, that of Balachowsky (1942). Modern systematists (Balachowsky, 1942; Morrison, 1928; Silvestri, 1939) agree that the families grouped by Balachowsky as the section Margaroïdae comprise the least specialized of the coccids; they are set off from all others by the retention (with a few specialized exceptions) of abdominal spiracles in all stages and well developed compound eyes in the adult male. Cytological data confirm this to the extent that in the Llaveiini there persist species with a basically primitive meiosis in both sexes, sex chromosomes of the ancestral type, normal sex ratio, absence of parthenogenesis and hermaphroditism. The tribe Iceryini, so highly specialized in parthenogenesis and hermaphroditism, still betrays in general cytological features and low chromosome number a close affinity to the Llaveiini. Again in the single matsucoccine thus far studied the evolution of a compound sex chromosome mechanism has been superimposed upon an otherwise primitive chromosome cycle. On the other hand, the cytology of the Margaroïdae indicates either a very early branching within, or a multiple origin from, the ancestral stock, since each tribe sampled shows a unique line of cytological specialization. New evidence on the interrelations of the sub-sectional categories of the Margaroidae seems assured in as much as field data indicate that but a small minority of the existing forms are as yet known, and only a very few of

these have been studied cytologically. Indeed the cytological evidence now available already indicates the need of a revision of one family — the Margarodidae — of this section, as the following evidence shows.

In the Margarodidae the subfamily Coelostomidiinae is characterized by the retention of the adult female within the test of the preadult, and by the various morphological changes associated with this habit. On this basis *Nautococcus schraderae* must be placed in the Coelostomidiinae, and has so been described (Vayssière and Hughes-Schrader, 1948). But the cytological evidence reviewed in an earlier section shows Nautococcus to be a typical llaveiine, of the subfamily Monophlebinae. The tribe Llaveiini, cytologically considered, is a remarkably closely knit assembly of species. Identical chromosome numbers and sex chromosome mechanisms, a highly specialized meiotic division figure characteristically modified in each genus but of a unique type common to all, and a joint tendency to asynapsis which varies in the intensity of its expression but is identically regulated in all, bespeak for the Llaveiini an extremely close kinship. Now not only does Nautococcus share this specialized llaveiine cytological heritage, but in addition it has evolved jointly with the llaveiines Protortonia and Llaveiella the unique linear aggregate type of spindle in male meiosis.

Unfortunately, of the Coelostomidiinae exclusive of Nautococcus only *Marchalina hellenica* has been studied cytologically (Hovasse, 1930). This species, it will be recalled, is obligatorily parthenogenetic with a chromosome number of 18 — in contrast with that of 6 which characterizes alike Nautococcus and all the Llaveiini. Marchalina, moreover, possesses a type of symbiosis unique among coccids and lacks both symbiont organ and mycetocytes (see page 198). Nautococcus, on the other hand, conforms in mycetome, mycetocytes, and symbionts closely to the llaveiine type. Surely it is far more probable that the changes involved in the retention of the adult female in the test of the pre-adult have had an independent origin in different subfamilies than that so extensive and complicated a series of correlated cytological specializations should have evolved independently and with such precise paralellism. Obviously cytological data will be more than ordinarily useful in the now necessary revision of the Margarodidae; information on the chromosome cycle and especially the meiosis of the male will be prerequisite to such revision.

The cytological validity of the tribal unit Llaveiini of the subfamily Monophlebinae has been noted. The tribe Iceryini constitutes a similarly natural group with identical chromosome numbers and facultative haploid arrhenotoky in all. The transformation of the females into functional hermaphrodites in *Icerya purchasi* has clearly been superimposed on a pre-existing haplo-diploidy whose origin is probably coeval with the tribe.

Especially to be desired relative to the latter point are cytological data on the genus Gueriniella. As noted earlier no males are known in *G. serratulae;* if thelyotokous it must have diverged from the tribal stock prior to the establishment of haplodiploidy. This is the more likely since Gueriniella is taxonomically rather distinct from the other iceryine genera. On the other hand, it may prove to be another hermaphrodite, and one in which the complete elimination of males, partially realized in *Icerya purchasi,* has been consummated (see p. 154).

Of the 10 families grouped by Balachowsky as the Lecanoïdae, 14 species, representative of 3 families, have been studied cytologically. Of these, 13 species have in common a highly specialized male meiosis characterized by asynapsis, differential behavior in the two haploid complements, and suppression of sperm formation in one of the latter. A single common origin of so complex a mechanism is almost certain; for the 3 families involved, the cytological data thus demonstrate a close relationship and justify a common grouping. Of especial interest is the nonconforming species, Puto sp. of the pseudococcine subfamily of the Eriococcidae, for it alone among the forms studied, shows no trace of the male meiotic specializations of the other lecanoïds but retains a primitive chromosome cycle. This suggests that Puto may prove to be more widely separated from the other mealy bugs than is currently supposed. It also confirms the taxonomic evidence placing the pseudococcines closest of all lecanoïds to the margaroïd stem. In this connection a cytological investigation of *Phenacoleachia zealandica,* sole representative of the family Phenacoleachidae and least specialized of the Lecanoïdae, is greatly to be desired, since on taxonomic grounds it occupies a central position in relation to a radial differentiation of ortheziid, monophlebine, and pseudococcine stocks. The need for comparative cytological studies in the Eriococcinae in relation to possible tetraploidy has already been discussed (p. 183). Finally, since 6 additional families among the specialized Lecanoïdae remain cytologically unexplored there is no reason to assume that either the complete range or maximum degree of specialization within the section has yet been discovered.

The third major subdivision — the Diaspidoïdae — embraces the true scale insects; it is the largest in point of included species and morphologically the most highly specialized of the entire superfamily. Its cytology remains unexplored. A single exception is the thelyotokous strain of *Aspidiotus hederae.* The general type of parthenogenesis here encountered has undoubtedly originated independently many times within the Coccoïdea. Cytologically demonstrated cases are known in each of the other major sections also, in the Margaroïdae, *Marchalina hellenica,* and in the Lecanoïdae, several species of Lecanium. Breeding and collection data

indicate a far wider spread and more frequent origin. It is already clear (see pp. 144–145) that further work on the relation of parthenogenesis and racial and specific differentiation will be richly rewarded. In this connection, the establishment of the basic chromosome number in the Coelostomidiinae, discussed above, will be of particular interest. Should it prove to be 2n ♀ = 6, as in the Llaveiini, a triploid origin becomes possible for the obligatorily parthenogenetic *Marchalina hellencia.*

Although this review is primarily limited to chromosomal cytology, the actual and potential contributions to taxonomy of comparative studies of symbiosis must be briefly considered. Based on the work of Walczuch (1932) three major categories of symbiosis in coccids may be distinguished: (1) the symbionts inhabit special cells, the mycetocytes, which are aggregated in a definite organ, the mycetome (all Margarodidae examined except *Marchalina hellenica*, Pseudococcinae); (2) Mycetome absent, mycetocytes present and loosely associated with fat body (Ortheziidae, all Lecanoïdae except certain Lecanium and Tachardina species, all Diaspidoïdae examined); and (3) neither mycetome nor mycetocytes present, symbionts dispersed in fat tissue (Lecanium and Tachardina species) or inhabiting gut epithelium (*Marchalina hellenica*). The first two categories are further and complexly differentiated according to structure of mycetome, kind and number of kinds of symbionts, adaptations of host for transmission of symbionts, etc. Walczuch considers symbiosis to have progressed from commensalism in gut, through symbiosis in gut epithelium, to the evolution of mycetocytes and finally of a mycetome organ. She is thence forced to conclude that symbiosis in coccids is of multiple and relatively recent origin, — subsequent to the separation of existing subfamilies and even tribes. On the contrary, it is more probable from its prevalence among Homoptera in general that symbiosis antedates the separation of the Coccoïdea from the ancestral stock. The course of its evolution within the coccids has then, as with so many other morphological systems in this group, been one of progressive loss and degeneration of parts. On this basis the data on symbiosis confirm the primitive status of the family Margarodidae. The single known exception, *Marchalina hellenica*, has lost mycetome and mycetocytes and has secondarily developed a new symbiosis with an organism inhabiting the gut epithelium. Whether this condition characterizes other Coelostomidiinae is not known; at any rate it confirms the justice of Morrison's (1928) removal of Marchalina from the Monophlebinae. The Ortheziidae have also lost the mycetome, but have retained mycetocytes — and this situation also obtains in the majority of Lecanoïdae and all Diaspidoïdae examined. Significantly, the pseudococcine group alone among lecanoïds retains the mycetome organ

— thus confirming the close linkage, cytologically so apparent in Puto, of lecanoïd and margaroïd stems.

The kind and number of kinds of symbionts frequently vary among closely related species. That these characters should be labile is not surprising. Loss of a symbiont might follow mechanical failure in infection of eggs and become established by migration to a region, or host plant, devoid of these particular organisms. Again, invasion of a new territory or new association of species might result in infection by new symbionts or potentially symbiotic organisms. Something of this sort must have occurred in *Icerya aegyptica* (Walczuch, 1932) which in Africa has two morphologically dissimilar symbionts while in India an otherwise indistinguishable strain of the coccid carries only one. Further comparative study of symbiosis in coccids is highly desirable; its potential value in taxonomy is already apparent and its possible role in the differentiation of geographic races should be investigated.

IX. Cytological Summary

The data of coccid cytology have thus far been discussed in reference to the analysis of the diverse mechanisms evolved in the various groups. Their bearing on general cytological problems other than mitosis and sex determination has been only incidentally indicated. In this section accordingly is attempted a brief summary of points of general cytological interest.

1. The diffuse nature of the kinetochore has been demonstrated by its direct effects on chromosome structure and behavior, namely: (1) absence of any primary constriction or differentiated organelle associated with spindle fiber attachment; (2) orientation of the whole body of the chromosome in the equator at metaphase; (3) production of chromosomal fibers along the entire length of the chromosomal unit; (4) parallel anaphasic disjunction; and (5) the mitotic potency of all fragments of a chromosome. As additional indirect effects, further detailed below (see 6), it modifies bivalent form and orientation; (6) facilitates the establishment of asynapsis; and (7) the autonomous action of chromatids and their constituent units. That it has also made possible evolutionary increase in chromosome number is highly probable as witness (a) healing of broken chromosome ends in somatic cells after X-ray induced fragmentation and mitotic ability of fragments (p. 132), and (b) evolution of a compound X chromosome mechanism in an XO group (p. 190).

2. Autonomy of the chromosome and of its constituent units is shown in: (1) asynchronous coiling cycles of the chromosomes in spermatid and sperms of iceryines; (2) initiation by a particular chromosome of the migration into the tail of the spermatid (in iceryines p. 159); (3) separation and

independent mitotic activity of the two chromatids of a chromosome, occasional in X of Llaveiella, and constant in all chromosomes in meiotic prophase of Protortonia; and (4) asynchronous anaphasic disjunction of chromatids (Nautococcus, and Protortonia), half chromatids and quarter chromatids (Llaveiella).

3. Multiple strand structure of chromosome: complete separation of constituent chromonematal coils (diffuse kinetochore) establishes a minimum value of 4 strands at metaphase, 2 at anaphase, in both somatic and meiotic chromosomes; occasional asynchronous disjunction of quarter chromatids indicates an 8 strand structure at meiotic metaphase (Llaveiella).

4. Pairing and association of chromosomes and chromosomal units: (1) parallel association of multiple and distinctly separated chromosomal units is shown in both somatic and meiotic metaphase chromosomes; (2) active pairing of homologous chromosomes is likewise not limited to univalent elements, since the 4 derivative chromatids of a given pair of autosomes, completely disjoined at first meiotic anaphase, reassociate in a parallel pairing of pairs at interkinesis (Llaveiella, Nautococcus). This is not "progeny pairing" but involves an actual moving together of the chromosomal elements. (3) Separated sister chromatids may reassociate in a common coil while in a relatively extended state (Llaveiella spermatid); and (4) an end to end association of chromosomes, involving terminal "attraction" is demonstrated in oogonial prophases of *Icerya purchasi* (p. 151), and in spermatid and sperm of iceryines (p. 159).

5. Structure and orientation of meiotic bivalents: effect of the diffuse kinetochore on bivalent structure is evident in separation of sister chromatids throughout their length, and in the absence of a localized "repulsion effect" such as is associated with the ordinary kinetochore. The terminal association between homologous chromosomes in a rod bivalent may break before metaphase and bivalent form be modified by shifts in the relative position of homologues. But whether attached or separate, each chromosome orients independently; there is no co-orientation of homologous kinetochores above and below the equator in the coccid bivalent.

6. Sequence of division and segregation in meiosis: all non-crossover chromosomes and chromosome regions undergo equational separation in the first division. Homologous chromatids disjoin at first anaphase or earlier, reassociate in a secondary pairing at interkinesis, and the second division is reductional for non-crossover regions. The X chromosome also divides in the first and segregates in the second division in the Llaveiini, but this sequence is reversed in Puto and Matsucoccus.

7. Asynapsis is compatible with normal segregation in male Llaveiini through the regular division of asynaptic chromosomes at first anaphase and the secondary pairing of their homologous derivatives at interkinesis.

8. Segregation without prior synapsis may also be brought about by differential heteropycnosis and coiling cycles in the two haploid complements (male lecanoïds).

9. Formation of non-flagellate sperms from diverticulum of nucleus (pp. 159–162) is demonstrated.

10. Orientation of chromosomes in sperm (Iceryini, pp. 159–162): in the mature sperm the chromosomes are arranged in linear order, attached end to end. They are approximately 12 times as extended as in meiotic metaphase. A four-phase spiralization cycle is demonstrated in chromosomes of spermatid and sperm.

11. Polar bodies function in embryonic development and organ formation (pp. 155–156).

12. Cytology of a gynomonoecious hermaphrodite is described; gonial reduction is associated with male differentiation (pp. 148–154).

13. The following types of parthenogenesis occur: diploid thelytoky through suppression of synapsis and second division, and by fusion of second polar body with female pronucleus; haploid arrhenotoky.

14. Meiosis in haploid males is described (p. 157).

15. Coccid cytology in relation to mitosis is considered (pp. 185–189).

X. LITERATURE CITED

Balachowsky, A., *Ann. Ec. Agric. Grignon*, (Ser. 3) **3**, 34–48 (1942).

Buchner, P., Tier und Pflanze in intrazellularer Symbiose, 462 pp. Gebrüder Borntraeger, Berlin (1921).

Costantino, G., *Boll. Zool* (Turin) **9**, 115–123 (1938).

Crouse, H. V., *Anat. Rec.* **94**, 45 (1946).

Dickson, R. C., *Hilgardia* **13**, 513–522 (1941).

Dreyfus, A., and Breuer, M. E., *Genetics* **29**, 75–82 (1944).

Federley, H., *Soc. Sci. Fenn.* (Com. Biol. IX) **13**, 1–12 (1945).

Ferris, G. F., Atlas of the scale insects of North America. Stanford University Press, (1937).

Gabritchevsky, E., *Russk. zool. Zh.* **3**, 295–332 (1923).

Goldschmidt, R., Mechanismus und Physiologie der Geschlechtsbestimmung 251 pp., Gebrüder Borntraeger, Berlin, (1920).

Hovasse, R., *C. R. Acad. Sci., Paris* **190**, 1025 (1930).

Hughes-Schrader, S., *Z. Zellforsch.* **2**, 264–292 (1925).

Science **63**, 500–501 (1926).

Z. Zellforsch. **6**, 509–540 (1927).

J. Morph. **50**, 475–495 (1930a).

Ann. ent. Soc. Amer. **23**, 359–380 (1930b).

Z. Zellforsch. **13**, 742–769 (1931).

Biol. Bull., Woods Hole **69**, 462–468 (1935).

Biol. Bull., Woods Hole **78**, 312–337 (1940).

J. Morph. **70**, 261–298 (1942).

Biol. Bull., Woods Hole **87**, 167–176 (1944).

J. Morph. **78**, 43–83 (1946).

Hughes-Schrader, S., and Ris, H., *J. exp. Zool.* **87**, 429–456 (1941).
James, H. C., *Bull. ent. Res.* **28**, 429–461 (1937).
Proc. R. ent. Soc. Lond. (Ser. A) **12**, 73–79 (1938).
Trans. R. ent. Soc. Lond. **89**, 569–577 (1939).
Junker, H., *Arch. Zellforsch.* **17**, 185–259 (1923).
Kuwana, I., *Bull. Pl. Quarant. Sta., Yokohama* **2**, (1922).
Mahdihassan, S., *J. Indian Inst. Sci.* **9A**, 1–24 (1924).
Z. angew. Ent. **16**, 527–545 (1930).
McClintock, B., *Genetics* **26**, 234–282 (1941).
Menozzi, C., *Redia* **16**, 105–110 (1927).
Morrison, H., *Tech. Bull. U.S. Dep. Agric.* **52**, 1–240 (1928).
Oksala, T., *Ann. Acad. Sci. Fenn.* (Ser. A. IV) **4**, 1–63 (1943).
Östergren, G., and Prakken, R., *Hereditas, Lund* **32**, 473–494 (1946).
Parr, T., *Yale For. Sch. Bull.* **46**, 1–51 (1940).
Pierantoni, U., *Monit. zool. ital.* **21**, 294 (1910).
Z. wiss. InsektBiol. **7**, 322 (1911).
Arch. zool. Ital. **5**, 321–400 (1912).
Arch. Zool. (ital.), Napoli **7**, 27–49 (1914).
Piza, S. d. T., *Amer. Nat.* **77**, 442–462 (1943).
Pollister, P. F., *Quart. J. micr. Sci.* **80**, 127–152 (1937).
Prakken, R., and Müntzing, A., *Hereditas, Lund* **28**, 441–482 (1942).
Rhoades, M. M., and Vilkomerson, H., *Proc. nat. Acad. Sci., Wash.* **28**, 433–436 (1942).
Ris, H., *J. exp. Zool.* **90**, 267–330 (1942).
Biol. Bull. Woods Hole **85**, 164–178 (1943).
Schmieder, R., and Whiting, P. W., *Genetics* **32**, (1947).
Schrader, F., *Biol. Bull. Woods Hole* **40**, 259–270 (1921).
Z.i.A.V. **30**, 163–182 (1923a).
Biol. Bull. Woods Hole **45**, 279–302 (1923b).
Arch. Zellforsch. **17**, 45–62 (1923c).
Z. wiss. Zool. **134**, 149–179 (1929a).
Psyche, Cambridge **36**, 232–236 (1929b).
Ann. ent. Soc. Amer. **23**, 126–132 (1930).
Z. wiss. Zool. **138**, 386–408 (1931).
Cytologia, Tokyo **6**, 422–430 (1935).
Evolution **1**, 134–142 (1947)
Schrader, F., and Sturtevant, A. H., *Amer. Nat.* **57**, 379–381 (1923).
Schrader, F., and Hughes-Schrader, S., *Z. wiss. Zool.* **128**, 182–200 (1926).
Schrader, F., and Hughes-Schrader, S., *Quart. Rev. Biol.* **6**, 411–438 (1931).
Scott, A. C., *Z. Morph. Ökol. Tiere* **33**, 633–653 (1938).
Silvestri, F., *Compend. Ent. App.* **1**, 618–860 (1939).
Smith, S. G., *Sci. Agric.* **21**, 245–305 (1941).
Suomalainen, E., *Ann. Acad. Sci. Fenn.* (Ser. A.) **57**, 1–30 (1940).
Thiem, H., *Z. Morph. Ökol. Tiere* **27**, 294–324 (1933).
Thomsen, M., *Z. Zellforsch.* **5**, 1–116 (1927).
Trans. 4th int. Congr. Ent. Ithaca, 18–24 (1929).
Troedsson, P. H., *J. Morph.* **75**, 103–147 (1944).
Vayssière, P., *Ann. Epiphyties* **12**, 197–382 (1926).
Vayssière, P., and Hughes-Schrader, S., *Mem. Mus. Hist. Nat., Paris*, in press (1948).
Walczuch, A., *Z. Morph. Ökol. Tiere* **25**, 623–729 (1932).

White, M. J. D., Animal Cytology and Evolution. 375 pp. Cambridge University Press, London (1945).
J. Morph. **79,** 323–369 (1946a).
Amer. Nat. **80,** 610–618 (1946b).
J. Morph. **80,** 1–24 (1947).
Whiting, P. W., *Quart. Rev. Biol.* **20,** 231–260 (1945).
Winkler, H., Verbreitung und Ursache des Parthenogenesis in Pflanzen-und Tierreiche. 231 pp. Gustav Fischer, Jena (1920).
Wolf, E., *Chromosoma* **1,** 336–342 (1939).

The Bearing of the New Systematics on Genetical Problems
The Nature of Species

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CONTENTS

I. Results of the New Systematics

The changes in the techniques and concepts of taxonomy during the past fifty years have made this science a valuable ally of genetics in the study of evolution. This new systematics has had a significant impact on the development of certain fields of genetics, in particular that of population genetics, and genetics in turn has profoundly influenced the thinking of the new systematist. Both fields have reacted upon each other in so many ways that it is often impossible to trace a particular method or concept back to its original source. There is a striking similarity between recently published works from the field of population genetics, like that of Dobzhansky (1944a) on the distribution of gene arrangements in the third chromosome of *Drosophila pseudoobscura*, and from the field of taxonomy, like that of Moore (1944) on geographical variation in *Rana pipiens*. In view of the close relationship of the two fields it is natural that the geneticist should wish to keep informed of the progress in the field of systematics. The present review deals with some aspects of animal taxonomy.

A number of developments are responsible for the change from the

orthodox to the new systematics. They can perhaps be grouped best under three headings:

A. *The Modification of the Linnaean Species.* The species of Ray and of Linnaeus were those of naturalists studying the faunas and floras of local areas. Such species are on the whole well defined and sharply delimited. Since they have no extension either in space or in time, they can be said to be without dimensions, to borrow a term from the field of geometry (Mayr, 1946c). The explorations of the post-Linnaean period added the dimensions of latitude and longitude to the Linnaean species.[1] The result was the development of a new species concept, that of the geographically variable or polytypic species, and the adoption of a trinomial nomenclature.

B. *The Recognition of the Population as the Basic Taxonomic Unit.* The individual was considered the basic unit by most of the older naturalists. The type specimen was thought to be "typical" and other specimens that did not agree with the type were described as varieties. Eventually, however, it was realized after painstaking genetic and biometric analysis that in sexually reproducing animals no two specimens (except identical twins) are exactly alike. Thus no specimen can be considered as "typical." What are "typical" are merely the statistical constants of the population to which a given individual belongs, for every local population can be defined at a given time by definite means and standard deviations in quantitative characters and by frequency percentages in qualitative characters. The population thus becomes the basic unit of taxonomy and the entire gene reservoir of an interbreeding local population forms the material basis of evolution.

C. *The Replacement of the Morphological Species Definition by a Biological Definition.* Morphological species definitions are based on the degree of morphological difference between two forms. Biological definitions emphasize the completeness of the gap ("distinctness") between species rather than the degree of difference. This is measured by the presence or absence of interbreeding ("gene exchange") with other populations.

The increasing penetration of these three changes through the whole field of taxonomy has had far reaching effects. The evidence for this has been presented in a number of recent reviews (Huxley, 1940, 1942; Mayr, 1942). These volumes summarize most of the relevant literature on animal taxonomy up to the year 1942. To avoid repetition only a few references will be made to papers referred to in these reviews.

Activity in the field of systematics has increased since 1942, but the

[1]The addition of the dimension of time in the field of paleontology, and its impact on taxonomic technique, will not be considered in the present discussion.

emphasis has somewhat shifted to a new set of problems. This indicates that considerable agreement has been reached on some of the earlier findings. The following seven statements incorporate some of the views now most widely held among animal systematists.

(1) Animal species have reality in nature and are well defined except in borderline cases.

(2) It is preferable to define species not on a morphological but on a biological basis, using reproductive isolation as a criterion. Species can then be defined as "groups of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups."

(3) Species are often composed of populations which are sufficiently distinct to be recognized as subspecies or geographical races. Species that consist of several subspecies are called polytypic species.

(4) The genetic differences that exist between the geographical races of a species may affect ecological preferences as well as morphological and physiological characters. In fact, all the characters of which species differences are composed are known to be subject to geographical variation.

(5) Wherever extrinsic factors cause a retardation or interruption of gene flow between portions of a species (geographical or spatial isolation), these subdivisions of the species tend to drift apart genetically. The rate of this change is different in different species.

(6) If the isolation is sufficiently complete and lasts sufficiently long, it will permit the evolution of isolating mechanisms, which will inhibit the interbreeding of the two daughter species after the elimination of the extrinsic isolating factors.

(7) Except in borderline cases there is a "bridge-less gap" maintained by isolating mechanisms between species that co-exist at the same locality ("sympatric species").

The majority of present workers are inclined to consider these seven statements as well substantiated, but a minority disagrees with some of them.

Other aspects of taxonomy are being studied actively at the present time, but have not yet reached that state of maturation which permits the formulation of generally acceptable statements. The three most active fields of current systematics are perhaps the ones which concern themselves with the following subjects:

(A) The nature and definition of species (the species attributes).
(B) The geographical structure of species (intraspecific variation).
(C) Discontinuities between species

1. Origin
2. Maintenance (isolating mechanisms)
3. Breakdown (hybridization).

All these fields are of vital interest to the geneticist, but not all of them are ripe for a review. So far as the structure of species is concerned, much new material has been published in recent years, but little that would permit the establishment of generalizations not contained in the reviews of Huxley (1942) and Mayr (1942). The study of isolating mechanisms and of hybridization between natural populations is pursued so actively at the present time that a complete review would seem premature. A survey of recent work on the origin of discontinuities was published separately (Mayr, 1947). It was therefore decided to concentrate in this review on a discussion of the nature of species.

II. The Definition of Species

The species is the most important working unit of the taxonomist, and it is therefore natural that numerous attempts have been made to find out what species really are and how they can be defined. Hundreds of species definitions have been the result of these efforts. I formerly (Mayr, 1942) grouped them in four categories, morphological, genetic, sterility, and biological definitions. It has since become apparent that there is some overlap between these four groups and that there are basically only two: "morphological" species definitions based on the degree of difference, and "biological" species definitions based on the amount of gene interchange, in other words, on the degree of reproductive isolation.

On the whole, there is a surprising unanimity among recent authors in the endorsement of a biological species concept. Among the dissenters seem to be Thorpe (1940), who calls many morphologically similar species biological races, and Sturtevant (1942), who states, "Distinct species must be separable on the basis of ordinary preserved material. This is in order to make it possible for a museum man to apply a name to his material." Consistent with this concept Sturtevant (1944) continues to refer to *Drosophila persimilis* Dobzhansky and Epling as race B of *D. pseudoobscura*.

The reasons for the gradual shift from the morphological to the biological species definition are manifold. Unconsciously the strongest is probably the philosophical unsoundness of the reliance on morphological difference. Species are products of nature and not specially made for the convenience of the "museum man." This is fully realized by the taxonomists themselves who to an ever-increasing extent endorse the biological species concept, even in cases where morphological differences are absent (*e.g.* M. Bates, A. Emerson). Recently proposed biological species definitions are by and large variations of a single theme. Emerson (1945) defines the species as "an evolved or evolving, genetically distinctive, reproductively isolated, natural population." The clause "evolved or

evolving" is unnecessary in a species definition. Neither is the phrase "genetically distinctive" necessary because there are differences in gene frequencies between all natural populations (many of which are not species) and because lasting reproductive isolation is unlikely, if not impossible, without genetic differentiation. Finally, most species consist not of a single, but of many populations, if we accept for population the now almost universally accepted definition as a local interbreeding unit. Mayr defines species simply as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups" (Mayr, 1942). In cases where occasional hybridization occurs it may be advisable to accept a more rigid definition along the lines of the one proposed by Mainland (1942): "A species is an actually or potentially interbreeding array of forms whose net mutation rate is greater than the actual or potential gene interchange with other arrays of forms." For a detailed discussion of criteria for vertebrate subspecies, species, and genera see Bogert *et al* (1943). For a discussion of the general principles of classification see Simpson (1945).

III. The Meaning of the Species

A species definition is merely the verbalization of a species concept. Species concepts, in turn, are based on the study of species. To many of the old fashioned museum taxonomists the "species" was an aggregate of museum specimens and it was easy for these workers to define the species in terms of the morphological characters of these specimens. They overlooked that in doing so they unconsciously made the species a human artifact. They did not say the species is a unit of nature, rather they said the species consists of specimens which conform to certain morphological criteria selected by me, the species is what I consider a species. The manifold difficulties of this subjective species concept led to a revaluation of the question: "What is a species?"

This question can obviously not be answered completely merely through a study of dead specimens. Species are aggregates of natural populations and can be fully understood only if at least some of them are studied as living populations. If two or more species of the same genus from a given locality are compared, it is found that in nature they are usually clearly delimited against each other. These well-defined units, the species of the naturalist, differ from each other in a number of attributes. There are various ways to classify these differences. The museum taxonomist may distinguish between quantitative and qualitative characters, or between diagnostic and not diagnostic characters. The general biologist may distinguish between morphological, physiological, and ecological attributes. These classifications may be quite satisfactory for

practical taxonomic purposes, but they completely leave out of consideration the relation any of these attributes may have to the maintenance of specific distinctness. *There are species characters that help to maintain the isolating gap between species, and others that are neutral in this respect.* Even though it is often difficult and sometimes impossible to decide in which category to place a certain character, it shall be attempted in the following section to classify species characters according to the role they play in maintaining species gaps.

IV. The Relation of Species Differences to the Maintenance of Isolation

1. *Neutral Characters*

Most of the morphological characters studied by the taxonomist are neutral in regard to the maintenance of isolation between species. The number of such morphological differences, even between closely related species, may vary strikingly from case to case. There are few or none in the case of sibling species, while other species pairs may differ in nearly every comparable pair of characters. "In addition to such morphological characters, there exist all sorts of physiological differences, particularly threshold differences and rates of growth and development, as well as inherited ecological adaptations and psychological reaction norms, such as differences in instinctive behavior." (Mayr, 1942, p. 18.)

In order to strengthen his species diagnoses, the taxonomist is paying more and more attention to features of the internal anatomy. Gordon and Benzer (1945), for example, describe specific differences in the gonopodial skeletons of allopatric species of Xiphophorine fishes (Platypoecilus and Xiphophorus).

The chromosome morphology is used by the plant taxonomist to an ever increasing extent. Karyology seems to be equally promising in many genera and families of animals. Dobzhansky, Patterson, and Sturtevant, as well as several other authors, have made substantial contributions in recent years to our knowledge of chromosomal variation in Drosophila. Such closely related species as *Drosophila pseudoobscura* and *D. persimilis* are diagnosed more easily by their chromosome configuration than by any other feature. In the genus Sciara also the chromosomes have an excellent diagnostic value. In a study of the Finnish bugs of the family Lygaeidae all the genera and nearly all of the fifty-six cytologically investigated species could be identified by their chromosomes alone (Pfaler-Collander, 1941). An exhaustive summary of the field is given by White (1945). Some of these cytological differences interfere with chromosome pairing and are thus isolating mechanisms and not neutral characters.

Physiological differences between species seem to be even more numerous than morphological ones. This is, of course, not surprising since morphological characters are, after all, merely the visible expressions of physiological processes. Such physiological differences can be considered to be of adaptive significance, although usually neutral as far as the maintenance of isolation between species is concerned. Only few detailed comparisons of the physiological constants of closely related species have so far been undertaken. Growth rates and egg-hatching periods in mosquitoes (Hovanitz, 1946) and growth rates and temperature reactions in various species of frogs of the genus Rana (Moore, 1942a and b, 1946a and b) differ significantly.

A combination of two or three hemolysis constants is absolutely diagnostic for each examined species of the mouse genus Peromyscus (Levine, 1943). The amount of difference between the studied species *leucopus*, *gossypinus*, *truei*, and *eremicus* corresponds approximately with the degree of morphological distinctness. Fox (1945, 1946) found that the various species of Daphnia differ in the spectroscopy of their hemoglobins. Irwin and his collaborators (Irwin, 1946; Irwin and Cumley, 1943; Irwin and Cole, 1945; McGibbon, 1944) have shown that each investigated species of bird has peculiar serological characters. All this work confirms the view that many, if not most, proteins are species specific. For a summary of this field, see Landsteiner (1945).

2. *Characters that Promote Geographic Coexistence of Species*

The exact significance of many attributes of species is unknown. Very often these characters appear to be merely accidental by-products of the genetic divergence of completely separated species. Most physiological characters, as for example those relating to temperature tolerance, are adaptive in regard to the environment. However, there is still another set of attributes of species whose primary function it seems to be to promote the simultaneous coexistence of several closely related species at the same locality. A considerable selective premium is placed upon the perfection of such characters and they may therefore evolve more rapidly than adaptively neutral species differences. Such characters cannot be studied in dead specimens with the orthodox methods of taxonomy. The systematist to whom such species differences are of vital interest is thus forced to enlarge his methodological repertoire and include in it the study of ecology and the behavior of living populations in nature and, if necessary, the experimental study of species in the laboratory.

The various characteristics that permit closely related species to coexist at the same locality can be classified roughly into two categories:

those that prevent or reduce competition and those that prevent or reduce the amount of interbreeding (reproductive isolating mechanisms).

A. The Reduction of Competition

It is an old observation of naturalists that closely related species which coexist at the same locality differ more or less noticeably in their ecological requirements. The full significance of this observation was not understood until it was pointed out that such ecological differences have considerable selective value. Individuals of two species with identical ecological requirements would be subject to the same competition for space and food as if they were members of a single species. However, since the two species are genetically different, one of them will undoubtedly be slightly superior to the other in a given habitat. Natural selection will discriminate against the less efficient individuals and thus eventually eliminate the less efficient species. This was deduced mathematically by Volterra and proven experimentally by Gause (1934) and others. As a consequence of this chain of events, there is a selective premium on differences in the ecological requirements of closely related species. (It may be mentioned parenthetically that some of these ecological differences may serve simultaneously also as isolating mechanisms by reducing the number of contacts between potential mates.)

The effect of this competition can be demonstrated by a few simple illustrations. If one of two closely related species of seed-eating birds prefers on the average larger seeds than the other, there will be no competition between the two species for the largest and for the smallest seeds. They will be beyond the threshold of one or the other species. Other factors being equal, selection will favor those individuals of the one species that are best prepared to feed on the largest seeds, and those individuals of the other species that are best prepared to feed on the smallest seeds. Individuals which feed on the seeds that are accessible to both species are exposed to increased competition. Again, if of two species of birds that feed in the canopy of the forest, species A feeds between 5 and 22 feet above the ground and species B between 15 and 40 feet, there will be no competition between the two species at the vertical levels of 5–15 feet (only species A) and of 22–40 feet (only species B). So far there is only scanty direct proof for the assumption that populations are kept in check by competition for space and food. It has been demonstrated for Protozoa (Gause, 1934) and for certain grain infesting beetles. However, more quantitative data are needed to counter the criticism that has been raised (Diver, 1945). Indirect proof, both positive and negative, is abundantly available (Lack, 1944).

The fact that some species have become rare or extinct in the part of

the range invaded by a closely related species is such indirect proof. The fact that species have greater altitudinal range or ecological tolerance in areas where their range is not overlapped by related species is additional evidence. The most abundant evidence for competition, however, has been derived from a study of closely related sympatric species. It has been found that such species nearly always have noticeable, if not striking, differences in their ecological requirements. There seem to be a few exceptions to this rule, as recorded by Lack (1944) and Diver (1940). Test (1945) describes the ecological requirements of the 17 species of limpets (Acmaea) known from the coast of California. There are only two cases of interspecific competition. The one concerns the three species *A. conus*, *A. digitalis*, and *A. scabra* high in the intertidal zone, and the second the species *A. testudinalis scutum* and *A. limatula* between Santa Cruz and San Pedro. Dice (1942) finds little difference in the ecology of *Peromyscus nasutus* and of *P. truei*, although *P. nasutus* appears to be more tolerant. Detailed analyses are not available for any of these cases, and it is highly probable that the apparent identity of ecological requirements is the result of incomplete knowledge.

Attributes of this sort are not necessarily diagnostic, of the type: "Species A feeds on maple, species B feeds on oak." There is usually a slight or considerable overlap in the requirements of competing species, rather than either a clear-cut difference or complete identity. Lack records many such instances for passerine (1944) and non-passerine (1945) birds. The cormorant (*Phalacrocorax carbo*) and the shag (*P. aristotelis*) are quite similar in appearance, and nest together on the rocky coasts of Europe. However, they do not compete with each other for either nest sites or food. The cormorant feeds chiefly in the shallow waters of estuaries and harbors and takes mostly fish which live on or close to the bottom (pleuronectids, gobies, also shrimps). The shag feeds mostly at sea on free swimming fish (Ammodytes, clupeoids). Among the Galapagos finches closely related species differ mostly in bill size, an attribute correlated with the size of the food. "On Chatham Island, the large *Camarhynchus psittacula* does not occur, so it might be expected that a greater range of foods would thereby become available for the small species *C. parvulus*. It is therefore significant that the Chatham form of *C. parvulus* has a larger beak than any other island form of this species . . . ." (Lack, 1947). A similar increase in the size of the bill occurs in the medium-sized *Geospiza fortis* on islands where the large-billed *G. magnirostris* is absent. The larger islands of the Galapagos are inhabited by 7 to 10 of the 13 known species of finches that occur in the archipelago. On these islands each species occupies a specific niche. On the smaller, outlying islands only 3 to 4 species are found, while the other finches are absent. Their food

niches are either filled by different species or one species may take food which on the central islands is divided between two or three species. In both cases there are corresponding modifications in the size and the shape of the bill (Lack, 1947).

The literature on frogs and toads is replete with indications of ecological differences between closely related species. Closely related stream animals have only partially overlapping ranges. Some species extend farther into the headwaters and are absent in the lower reaches, while the reverse is true for others. Such data are available for planarians (Beauchamp and Ullyott, 1932), crayfishes (Hobbs and Marchand, 1943), mayflies, and other groups (Hesse, Allee and Schmidt, 1937). In grasshoppers Isely (1946) found that some species prefer as food herbaceous plants, others succulents, and still others harsh grasses. The striking ecological differences of the 17 Californian species of Acmaea (limpets) are described by Test (1945). Most species feed only on a single species of marine algae. Gregg (1945) found pronounced differences between the nest sites of the ants *Lasius neoniger* and *L. americanus*. They occur in both soil and logs, but *neoniger* is usually a soil, and *americanus* a log inhabitant.

Some information is available on Drosophila, but mostly of a general nature. For example, there is evidence of ecological differences between the species of the following groups: *affinis*, *funebris*, *virilis*, *repleta* (*mulleri*), but the analysis in all of these cases is incomplete. Wagner (1944) found definite differences in nutritional requirements of the two closely related species *Drosophila mulleri* and *D. aldrichi*, the larvae of which coexist in Texas in the ripe fruit of a species of cactus (*Opuntia lindheimeri*). A study of the yeasts and bacteria required by these two species of Drosophila shows: "that *aldrichi* utilizes the known microorganisms differently than does *mulleri*. This fact in itself shows that the two species are different physiologically, and inhabit two different ecological niches, although the niches may correspond as to position in space and time. It might be said that the competition between the two forms is mitigated by the differential utilization of the microorganisms present in the feeding substrate. This . . . is not an ecological isolating mechanism between *D. mulleri* and *D. aldrichi* . . . which — even though reproductively isolated — might not be able to exist together unless this slight difference existed in their nutrition."

Ecologically distinct species are not always necessarily sympatric. Where habitat differences coincide with topographic features, either a curious interdigitation results or a clear-cut allopatry. Johnson (1943) describes several such instances among the chipmunks (Eutamias) of California. The two species, *E. quadrivittatus* and *E. speciosus*, are very closely related and more or less allopatric, but "*quadrivittatus* was taken only in the vicinity of open forests of stunted limber pine and white-bark

pine on exposed and well-drained ridges and slopes near timber line, whereas *speciosus* was taken only in or near the dense stands of lodgepole pine in the damper and more sheltered basins." No intermediate specimens were taken in the various zones of contact. Similar geographical overlaps with habitat segregation occurs between *E. townsendi* and *E. quadrimaculatus* in the Northern Sierra, and between *E. townsendi* and *E. sonomae* (inner coastal region of northern California and southern Sierra). The literature is replete with similar cases and it is sometimes difficult to decide whether the ecologically divergent allopatric populations should be treated as species or as subspecies. In fact, many of them seem to be borderline cases. Furthermore, it is difficult to decide in some of these cases whether the two allopatric forms cannot invade each others' ranges because their habitat requirements are too similar or too different.

The recognition of the importance of ecological competition is of great value in the interpretation of the taxonomic status of closely related allopatric populations with contiguous ranges. Some authors treat such populations consistently as subspecies since they interpret the absence of overlap as indicating the absence of isolating mechanisms. Stresemann (1939) pointed out, however, that the lack of overlap in many of these cases indicates ecological competition between the respective species (see also Mayr, 1942, pp. 176, 250, 263). Although such populations have reached species level, as proven by absence of interbreeding in the zone of contact, they cannot invade each other's ranges for lack of pronounced ecological divergence. This situation is, for example, illustrated by the two European nightingales, *Luscinia luscinia* and *L. megarhyncha*, which meet along a line of more than a thousand miles of length, extending from Denmark through eastern Germany and Russia to Siberia. The two species overlap each other's ranges occasionally in the contact zone for a width of 20 to 50 miles, but are then ecologically separated. *L. megarhyncha* is found in the drier woods and gardens, while *L. luscinia* prefers wet, swampy woods. A range expansion of one species is compensated by an equivalent contraction of the other. No hybrids have been found along the extended zone of contact. The slight ecological differences between the two species do not permit extensive mutual invasions of the other species range, but is sufficient to give each species an advantage in its respective range. Cases such as this one of the nightingales — and there are quite a number of similar cases known — offer a particularly clear-cut illustration of the force of competition.

As Stresemann (1939) has pointed out, there is some correlation between the number of ecological niches in a given region and the number of sympatric related species that can be accommodated. The poverty of island faunas is partly due to this factor. Species which on mainlands

live side by side may be strictly allopatric on islands. Elton (1946) shows that the average number of species per genus in 55 animal and 27 plant communities is as low as 1.38 and 1.22. A rather constant and high percentage of genera with only a single species is found, the average being 86 per cent for animal and 84 per cent for plant communities. The number of species per genus is much higher in districts that are geographically defined and include numerous different habitats.

Summarizing these data it can thus be said that closely related species seem not to be able to coexist at the same place without having acquired differences in their ecological requirements. This can be proven experimentally and inferred by observation. Such observations show (1) that if closely related species occur in the same habitat, they either have different food habits or occupy otherwise slightly different niches, (2) that in most cases closely related species that occur in the same geographical area prefer different habitats, and (3) that closely related species sometimes have geographically exclusive ranges, indicating either the inability to invade each other's ranges for ecological reasons, or the absence of suitable habitats in the respective areas.

Multiplication of species, leading to the coexistence in time and space of several closely related species, is possible only if competition between such species is mitigated by the acquisition of differences in ecological requirements. The ability of evolving species to become sympatric is thus dependent not only on the origin of isolating mechanisms, but also on additional genetic differentiation that diminishes competition for food and space. In some instances these ecological differences have developed into auxiliary isolating mechanisms through impeding the meeting of potential mates from different populations. Such differences are thus highly important evolutionary mechanisms and deserve a greater attention from geneticists than they have received in the past.

B. Reproductive Isolation

Agents that prevent or inhibit the successful interbreeding of natural populations have been termed isolating mechanisms by Dobzhansky. A number of classifications of these isolating mechanisms have been proposed in recent years, all of which agree in essential scheme and merely differ in the emphasis on various factors. The most important division, from the evolutionary point of view, is between extrinsic factors, such as geographical barriers and conditioned behavior, which are completely reversible bars to crossing, and intrinsic factors which have a genetic basis. These latter (except sterility) had been strangely neglected by naturalists and experimental biologists alike until a number of recent authors, partic-

ularly Dobzhansky, called attention to their importance. Much additional information on isolating mechanisms has accumulated since their discussion by Dobzhansky (1941), Huxley (1942), and Mayr (1942).

It has been customary to subdivide reproductive isolating mechanisms into those which prevent crossing and those which reduce the success of crosses (inviability and sterility of hybrids, etc.). Recent work on isolating mechanisms in the genus Drosophila indicates that the two kinds of mechanisms intergrade almost without a break, and that copulation by no means always results in transfer of sperm nor the transfer of sperm in the fertilization of eggs. From the point of view of selection the crucial aspect is whether or not the isolating mechanism prevents the wastage of gametes. An isolating mechanism which prevents the transfer of sperm to the wrong kind of female is obviously much more efficient than a mechanism which permits the transfer of sperm, that is either killed in the tract of the female before it can fertilize any eggs or that produces more or less inviable zygotes. Natural selection will favor any mechanism that will prevent such wastage of gametes.

The subject of isolating mechanisms is too large to be treated fully in the present review. Only that material will be presented which is needed to throw light on the speciation process and the nature of species. Important contributions to this field are the Symposium on Isolating Mechanisms (Patterson, 1942), the papers of Dobzhansky and others on sexual isolation in Drosophila (Dobzhansky, 1944b; Dobzhansky and Mayr, 1944; Dobzhansky and Streisinger, 1944; Levene and Dobzhansky, 1945; Mayr, 1946a and b; Mayr and Dobzhansky, 1945; Wallace and Dobzhansky, 1946), of Muller (1942), Stalker (1942), Rendel (1945), Patterson and his students (Patterson, 1942; Patterson and others, 1942, 1944), and others.

Mechanisms Which Prevent the Meeting of Potential Mates. Various closely related species of chipmunks (Eutamias) are segregated in different habitats in the zone of contact (Johnson, 1943). The same has been described for numerous species of animals. In the toads, *Bufo americanus* and *B. fowleri*, Blair (1942) finds that where the two species overlap there is often a differential preference for breeding places: "*Bufo fowleri* utilizes ponds, sloughs, and large rain pools as breeding sites, but does not breed in the shallow puddles and brook pools which are so extensively used by *B. americanus*. *B. fowleri* breeds in the quieter waters of streams but *B. americanus* rarely or never does so. *Bufo fowleri* collected near Wilburton, Oklahoma, May 26, 1939, were all found breeding in a creek. *B. americanus* taken at the same locality April 8, 1940, were all breeding in shallow rain pools, and not a single toad was found along the creek which was only a few hundred feet from the pools." A preference for the headwaters

or the lower reaches helps to prevent interbreeding between fish species of the genera Platypoecilus and Xiphophorus (Gordon, 1947).

There are some observations available which throw light on the importance of such habitat segregation. Among two closely related species of sparrows of the Mediterranean region the House Sparrow (*Passer domesticus*) lives usually in villages while the Willow Sparrow (*P. hispaniolensis*) lives in willow thickets in valley bottoms. There is no interbreeding between them in the areas where such ecological segregation exists. However, the Willow Sparrow has invaded villages in a few regions and in these places it interbreeds freely with the House Sparrow (Meise, 1936).

Differences in the breeding seasons may also impede the meeting of individuals of different species, as mentioned above, for the two species of toads. A similar difference has been demonstrated by Moore (1942a) for various species of frogs (Rana). This seems to be a particularly effective isolating mechanism in certain closely related species of insects, but it is impossible to say without experiment to what extent it is reenforced by additional mechanisms. It must not be overlooked, however, that seasonal barriers seem to be totally absent in large groups of animals. Most closely related species of birds in the temperate zone have synchronous breeding seasons. Neither is there any sign of seasonal isolation in the many groups of animals which breed continuously throughout the warmer part of the year.

The time of day at which reproduction takes place may also be important. "Closely related species of fishes (*Pomoxis nigro-maculatus* and *P. annularis*), with similar food habits and general behavior, occur together in the Illinois and Ohio Rivers and a few interspecific hybrids are known. One (*P. nigro-maculatus*) shows a nocturnal activity rhythm and the other (*P. annularis*) is diurnal, indicating that these species are reproductively isolated through their different activity cycles. In the same family (Centrarchidae) the forms that hybridize freely have activity periods which are the same or overlap broadly" (Emerson, 1943). *Drosophila subobscura* is sexually inactive in darkness (Rendel, 1945), and this activity rhythm thus is an important partial isolating mechanism between this species and other species of the *obscura* group (*pseudoobscura, persimilis*) (Wallace and Dobzhansky, 1946).

Ethological Barriers. Copulation is preceded in most species of animals by an interplay between male and female which arouses the necessary amount of physiological readiness. It is during this preliminary phase that the courtship of not-conspecific individuals is usually broken off. It has been assumed by a number of modern authors that this premature termination of courtship is due to sudden species recognition and that

"psychological barriers prevent or reduce the frequency of matings between members of closely related species. The terms "species recognition" and "psychological barriers" signify a crude concept of the interplay between male and female, which has no reality. The term "recognition" implies consciousness and the ability to make judgments for which no evidence exists in Drosophila. Rather it must be assumed that the male stimulates the female by specific pre-copulatory displays and that the female reacts by specific responses indicating a state of receptiveness. This interpretation assumes that successful copulation is the result of a chain of interactions between specific stimuli produced by the male and adequate responses of the female which in turn stimulate the male" (Mayr, 1946b). An analysis of psychological ("ethological" is actually a better term) isolating mechanisms must thus be an analysis of the stimuli that produce readiness for copulation. Such stimuli may affect any of the sense organs, since they may be visual, auditory, olfactory, gustatory, or tactile factors. It is the field of animal psychology which is primarily interested in the study of these factors, but since they are important evolutionary mechanisms they are of concern also to the geneticist, systematist, evolutionist, and general naturalist.

The incompatibility of the courtship patterns of different species has been described by many naturalists and has been studied in some detail for several species of Drosophila. Stalker (1942) studied it in the cross between *Drosophila virilis* and *D. americana*, Mayr and Dobzhansky (Mayr, 1946b; Mayr and Dobzhansky, 1945) in *D. pseudoobscura* and *D. persimilis*, Dobzhansky (1946) in *D. willistoni* and *D. equinoxialis*, and Patterson and his co-workers (Patterson, 1942; Patterson and others, 1942, 1944) for various species groups of Drosophila. The ethological barriers are fairly efficient in most of these cases and only a limited amount of interspecific fertilization takes place if conspecific females are available. A. P. Blair (1942), however, showed that ethological barriers were weak in the two similar species of toads, *Bufo americanus* and *B. fowleri*. Males clasped females of either species indiscriminately and showed only a slightly greater positive response to the call notes of their own species. As stated above, the isolation between these two species seems to be maintained mainly by ecological differences.

The presence of ethological barriers is usually assumed if closely related species coexist at the same locality and do not or only infrequently display to each other and do not copulate. This indicates that the stimuli produced by either species are not sufficiently specific to produce the amount of physiological stimulation necessary for copulation. Of the two species, *Colias chimera* and *C. cunninghami*, which overlap in the equatorial Andes, Hovanitz (1945) reports: "Interspecific attempts to pair

were fairly common and were reciprocal but the sexual maneuvers did not last long and were not completed. No actual copulations nor signs of intermediate products were observed." The nests of the cyprinid fish *Nocomis micropogon* in which spawning takes place are used also by three other species of the same family, *Notropis cornutus*, *N. rubellus*, and *Campostoma anomalum*, but other mechanisms prevent or reduce hybridization (Reighard, 1943). Several species are frequently mixed in the mating swarms of Chironomidae (Diptera), where the density of these midges is high. Still there seems to be no evidence of interbreeding (Gibson, 1945). In a mixed flock of the bugs *Lygus pabulinus* and *L. pratensis* there was a great numerical excess of the males of *pabulinus*. When they were unable to find females of their own species, they attempted, although unsuccessfully, to copulate with females of *L. pratensis* (Kullenberg, 1946). The isolating mechanisms between two species of bed bugs (*Cimex lectularius* and *C. hemipterus*) were studied by Omori (1939). Blair and Howard (1944) tested sexual isolation between three subspecies of the two closely related species *Peromyscus maniculatus* and *P. polionotus*. The natural ranges of these two species do not overlap. A male and female of each of two forms were placed in a battery of four connected cages and pair formation and reproduction observed. Counting as pairs those individuals that were found together in the sleeping cages the following combinations were scored. Of *Peromyscus maniculatus blandus* and *P. polionotus leucocephalus*, 394 intraspecific pairs were found and only twenty-one (= 5.1 per cent) interspecific pairs. With the *Peromyscus maniculatus blandus* and *P. p. albifrons* combination 139 intraspecific pairs were found and sixty-three (= 31 per cent) interspecific pairs. However, no hybrids were produced by the interspecific pairings, even though the cross is possible when no choice of mates is given.

How effective conditioned behavior is as isolating mechanism in animals is still in dispute. In man it has sometimes been fairly effective where different racial groups have come into secondary contact (Darlington, 1943).

Comparative Ethology. In the same way as morphological and anatomical characteristics change from species to species and from genus to genus, thus supplying material for a comparative study, so do behavior patterns. It may be too early to speak of a science of Comparative Ethology, but beginnings have been made in the analysis of the unit elements of which behavior patterns of some animal groups are composed and to compare their evolutionary modification from species to species. This was done by Lorenz (1941) for most species of river ducks (Anatini), by Wing (1946) in far less detail for the North American grouse (Tetraonidae), and by Spieth (1947) for the species of the *Drosophila willistoni* group. It

has been found that the behavior pattern is on the whole composed of homologous elements within a given taxonomic group, but that there is great variety in the manifestations of these elements, and that many of the modifications are species specific.

Incomplete and Complete Copulation without Fertilization. The chain of events leading from sexual display to the fertilization of the eggs can be broken at a number of places. Each of the possible breakage points is a potential isolating mechanism.

Mechanical difficulties may prevent the success of an attempted copulation, if structural differences prevent the male from inserting his genitalia into the genital opening of the female. Such mechanical isolation was considered at one time of great importance, at least among insects. The striking differences in the sexual armatures even between closely related species seemed to support this interpretation. Recent observations (Dobzhansky, 1941), however, indicate that this factor is much less important than formerly thought. Beheim (1942) shows that hybridization is even possible between species of carabid beetles with strikingly different sexual armatures. In some cases the post-copulatory separation of the sexes is difficult in interspecific crosses, as shown for Drosophila first by Sturtevant (1915) and confirmed by Stalker (1942) for the cross *D. virilis* with *D. americana.* The fit of the male hemipenis in the female cloaca is so perfect in some species of snakes that these structures may function as isolating mechanisms (Pope, 1941).

The amount of sperm transferred seems to be another factor contributing to reproductive isolation in the genus Drosophila. The ventral receptacles of females of *Drosophila pseudoobscura* and *D. persimilis* seem to be on the average less filled with sperm after an interspecific mating than after an intraspecific mating. Less offspring are thus produced by a heterogamic mating than by a homogamic one. This counter selection is enhanced by the lower viability of foreign sperm in the female genital tract.

Recently Patterson (1946) discovered a most remarkable isolating mechanism in the genus Drosophila. The vagina of the female of some species swells up to two or three times its normal size following copulation in certain matings between different species and this is followed by a dying off of the sperm. A reaction between male and female proteins seems to be involved.

Hybrid Inviability and Sterility. It cannot be the task of this review to survey the rapidly growing amount of information on this vast subject. Merely a few facts shall be mentioned which appear to be notable advances of the field. One is the discovery in protozoa of what corresponds to hybrid inviability in higher animals. Jennings and Opitz (1944), and

Sonneborn and Dippell (1946) found that if conjugation took place between certain "varieties" of the *Paramecium bursaria* and *aurelia* groups the crosses resulted either in the death of both conjugants or gene exchange was prevented by other means.

Lamy (1943) showed that the amount of interspecific sterility was strikingly different in a number of laboratory stocks of *Drosophila pseudoobscura* and *D. persimilis.* The greatest amount was found in stocks that had been longest in the laboratory. Her results indicate with what rapidity such sterility genes may become fixed under favorable conditions. Selection for sterility was not involved since the stocks had been selected for certain marker genes, as incidental by-products of which the sterility had developed.

The Significance and Operation of Isolating Mechanisms. The above recorded studies of isolating mechanisms permit a number of generalizations. First of all, that there is great diversity in the factors that prevent hybridization between individuals of different species. The potential mates may be segregated either ecologically or through differences in courtship and copulation or through various degrees of sterility and hybrid inviability and sterility. All the recent evidence shows that in the separation of a given pair of species many, if not most, of these mechanisms are somewhat involved. In cases where the reproductive isolation of two species under experimental conditioning is only partial, as for example between *Drosophila pseudoobscura* and *D. persimilis*, or *D. virilis* and *D. americana*, it can be shown that an unexpected number of factors have a minor effect on the reenforcement of this isolation: visual, auditory, olfactory, gustatory, and tactile clues, age and temperature, and the previous experiences of the individual. Reproductive isolation is thus not a simple lock and key mechanism that is controlled by a few genes. Although there may be and usually are a few decisive factors, they are usually modified and supplemented by many minor ones. This multiplicity of isolating mechanisms is of obvious selective advantage. A single factor might efficiently separate two species. But more than a single mechanism is required to separate a species from all other related species. Furthermore, it may be genetically easier to build up a multiple, mutually reenforcing mechanism than a single factor all-or-nothing barrier.

Within a given taxonomic group there is on the whole a certain amount of parallelism between the degree of morphological difference and the development of the isolating mechanisms. It is this correlation which the systematist utilizes to determine the taxonomic status of allopatric populations. However, the occurrence of sibling species on one hand and of strikingly distinct interbreeding subspecies on the other, prove that the correlation is not always very close.

The correlation between the potencies of the various isolating mechanisms is even less close. This is particularly true for sterility and ethological isolation. Many species of birds as, for example, Mallard (*Anas platyrhynchos*) and Pintail (*A. acuta*) produce in captivity fully fertile hybrids, but do not cross in nature in the vast area where their ranges overlap. Two subspecies of *Drosophila pallidipennis*, on the other hand, lack sexual isolation, but produce sterile F_1 males (Patterson and Dobzhansky, 1945). The horse-donkey cross is another such case of a sterility barrier, without fully developed ethological isolation.

If two temporarily isolated portions of a species have developed no incipient isolating mechanisms they will freely interbreed after the removal of the temporary barrier. Speciation is thus always preceded by the origin of potential isolating mechanisms. This consideration makes it obvious that subspecies of a species may differ in characters which may be potential isolating mechanisms. A number of recent investigations fully confirm this. In *Drosophila prosaltans* there is a definite north-south sequence in mating preference (Dobzhansky and Streisinger, 1944). Geographic strains of *D. sturtevanti* show incipient sexual isolation, but there is no strict geographical regularity. Some strains coming from far distant localities show less aversion to interbreeding than strains from less remote localities (Dobzhansky, 1944b). In *D. pallidipennis* there is sterility between populations from distant localities, but little sexual isolation (Patterson and Dobzhansky, 1945). Geographical variation of mating preference has also been found in *D. americana* (Stalker, 1942) and *D. repleta* (Wharton, 1942) and of sterility in *D. macrospina* (Mainland, 1942). In fact, all the available evidence indicates that a certain amount of either sexual isolation or of sterility or both is nearly always found in the genus Drosophila when geographically distant populations of a species are compared. Moore (1946b) crossed individuals of peripheral populations of the frog *Rana pipiens* and found much hybrid inviability.

It would be an error to consider such manifestations of the existence of incipient isolating mechanisms as proof of completed speciation. The populations involved in these experiments are as a rule completely connected with each other by interbreeding-intergrading populations. Rather these experiments confirm the *a priori* assumption that the development of incipient isolating mechanisms in geographically distinct populations is a prerequisite of geographical speciation. Two populations can invade each other's ranges without losing their identity only *after* such mechanisms have developed. Hybrids, except under special ecological conditions, have survival value which is inferior to that of the parental populations. Thus, there is a selective advantage to prevent the destruction (by recombi-

nation) of favorable stabilized gene combinations. Natural selection will thus tend to improve imperfect isolating mechanisms.

V. Difficulties of the Species Concept

Well defined species are thus characterized (1) by morphological and physiological differences, (2) by reproductive isolation, and (3) by ecological differences. Nobody will hesitate to use the term species for natural populations that fulfill each of these three requirements. However, in addition to such clearly defined species, there are many natural populations which either do not show all three distinctions, or for which, unfortunately, the available information is insufficient. These are the cases in which any species definition is faced with difficulties. At the same time, these are the very cases which have shed most light on the true nature of species and their origin.

If such populations are classified on the basis of three sets of information (geographical relationship, presence or absence of morphological difference, and of reproductive isolation), a table with eight squares can be constructed (see Table 1). It is assumed in this table that all informa-

TABLE 1

Individuals are:	Not reproductively isolated	Reproductively isolated
Morphologically identical Sympatric	1. Same population	5. Sibling species
Allopatric	2. Same subspecies	6. Sibling species
Morphologically different Sympatric	3. Individual variants of the same population (polymorphism)	7. Different species
Allopatric	4. Different subspecies	8. Different species

The assumption is made in squares 6 and 8 that intergrading populations are absent.

tion is not only available, but also properly weighed. The difficulties and errors of the taxonomist occur where this assumption is not fulfilled. For example, disregard of lack of reproductive isolation and overemphasis of morphological difference leads to the erroneous recognition of populations in square 3 and 4 as full species. It leads likewise to a non-recognition of the species status of the sibling species in squares 5 and 6. This is wrong because the ethological, ecological, and physiological characters that guar-

antee the maintenance of species as separate entities can be called species characters with much greater justification than morphological characters many of which are merely incidental by-products of genetic divergence. "Too great an emphasis on morphological distinctions as species criteria leads to results that are plainly untenable. One would have to break living mankind into five species that are not at all isolated reproductively, and yet consider as single "species" some groups of clearly separate species of Drosophila" (Mayr and Dobzhansky, 1945).

Giving due weight to the importance of reproductive isolation, the difficult situations can be decided by the taxonomist as follows:

1. Morphologically distinct, sympatric, but not reproductively isolated individuals (square 3) are members of a single interbreeding population (either polymorphic variants or ecophenotypes).
2. Morphologically identical, either sympatric or allopatric, reproductively isolated individuals (squares 5 and 6) are members of different populations (sibling species).
3. Morphologically distinct, allopatric populations of which the degree of reproductive isolation is unknown may be either subspecies or allopatric species (squares 4 or 8).

These three groups of difficulties in species determination will be discussed in the following sections.

Morphologically Distinct Variants within a Single Population. Male and female, immature and adult, larva and imago, and other stages and phases in the life of a single species are often morphologically strikingly different. The synonymies of the taxonomist contain many examples where such stages or forms of a species were originally described as separate species. Naturally, as soon as it is realized that two or more of such "species" are members of a single interbreeding population, they are considered conspecific regardless of their striking morphological differences. The same is true also for individual variants of polymorphic species, many of which were described as full species before their true nature was ascertained. Numerous instances of this have been recorded by Mayr (1942, pp. 72–85), Ford (1945), and others. Only a single case will be discussed here, since it offers a particularly clear-cut illustration. There are two king snakes (Lampropeltis) in southern California, a ringed and a striped one. The ringed one was described in 1853 as *boylii* by Baird and Girard, the striped one in 1835 as *californiae* by Blainville. The two forms were considered good species for nearly 100 years until Klauber (1936, 1939) showed that both types of snakes occur in the offspring of either kind of female. Even though the absence of "blending" between the two forms was used to argue in favor of their specific distinctness (Smith, 1943), it was shown by Mayr and Dunn (1944) that simple Mendelian inheritance

is involved. The figures calculated with the Hardy-Weinberg formula show that the progenies of ringed mothers occur about in the proportions expected on the assumption of random mating and a single gene difference. Even though there is a deficiency of ringed offspring among the broods produced by striped mothers, there can be no doubt that the striped form is merely an allele of the ringed pattern, and not a separate species. Similar cases can be found in all groups of animals and many of the synonyms which "grace" taxonomic monographs owe their existence to the error of recognizing species on the basis of single gene differences.

1. *Sibling or Cryptic Species Among Animals*

Pairs or groups of morphologically nearly or completely identical species have been termed sibling species (Mayr, 1942, p. 151). Their existence is usually revealed only if they coexist at the same locality, and in my original definition I had therefore limited the definition to sympatric species. This is, however, not necessary since it is feasible with modern techniques to test the conspecificity of allopatric populations. It is not certain, for example, that *Drosophila willistoni* occurs at Teffe, upper Amazon, from where the indistinguishable, but sexually isolated species, *D. equinoxialis*, was described (Dobzhansky, 1946). Still the two species are obviously to be considered sibling species. Such morphologically similar pairs or groups of species have been described in the recent literature with increasing frequency. They are found in nearly all systematic categories, although they seem to be decidedly more frequent in some than in others.

Chapin (1944) has recently added a number of species among birds to the list of those previously known. Among the African bulbuls such pairs are *Phyllastrephus icterinus* and *P. xavieri*, *Phyllastrephus strepitans* and *P. terrestris*, *Andropadus curvirostris* and *A. gracilis*, *Baeopogon indicator* and *B. clamans*. In each case one species is distinctly larger than the other while differences in coloration and other features are negligible. Even though these species pairs are sympatric, there is evidence of ecological divergence. The analysis of the sibling species complex of cave swiftlets (Collocalia) has not made much progress in recent years. This group remains the last unanalyzed genus of birds. Recent investigations (Mayr, 1945) indicate that in the Philippines there are at least two more species than admitted by the latest checklists.

The discovery of such sibling species is possible only in groups that are either very well known taxonomically, as for example birds, or to which particularly refined methods have been applied. Such methods may be either biometric (as particularly among fish), or cytogenetic (as, for example, in Sciara and Drosophila) or combined taxonomic-ecological

(as, for example, in Anopheles). Elaborate studies of this sort are generally carried out only in groups that are medically (*e.g.* Anopheles) or otherwise of special significance. It is for this reason as yet impossible to state how widespread is the occurrence of sibling species, or to give the approximate percentage of sibling species in various orders. Furthermore, many situations have been published in the literature which cannot yet be classified because they are still incompletely analyzed. Kennedy (1943), for example, found two groups of whitefish (*Coregonus clupeaformis*) in Lake Opeongo, Ontario, which are distinguished from each other by the size at which they mature. The size distribution of mature individuals of both sexes is bimodal, the modes coming at 120 mm. and 240 mm. No overlap of size exists between fish of equal age. There are slight but significant differences in the number of scales and gill rakers, but no differences in proportions. The "dwarfs" grow more slowly and for a shorter season, mature when younger, and have a shorter life span. The vertical distribution during the growing season is the same for the two types, hence the difference cannot be due to environmental conditions. It is unknown whether reproductive isolation exists between the two types and nothing can therefore yet be said on the taxonomic and evolutionary status of these two kinds of fish.

Physiological races have been described particularly among insects. However, as A. Emerson (1945) points out, most of these so-called physiological races answer all the criteria of good species. This is true for the species of the *Anopheles maculipennis* complex and for the tree crickets of the *Nemobius fasciatus* group. Recent work in several orders of insects fully confirms these conclusions.

In the Hymenoptera, differences between closely related species of ants are often particularly slight. This is one of the reasons for the complication of ant nomenclature. In the past, myrmecologists did not dare to recognize barely distinguishable sympatric forms as full species and were thus forced to resort to a complicated quadrinomial nomenclature of races, subspecies, and varieties. Gösswald (1941) contributed to an analysis of the *Formica rufa* group, and E. V. Gregg (1945) carefully compared two of the American species of the *Lasius niger* complex. She comes to the conclusion that the two so-called varieties *neoniger* and *americanus* of *Lasius niger* are good species. In addition to a number of ecological differences between the two forms, it was found that actually no morphological overlap existed. All of twenty workers of *neoniger* had hair on the scape of the antennae and on the tibiae where not one hair was found in *americanus*. The differences in the number of hairs on coxae, trochanters, and femora were statistically significant. At least in the Chicago area where this investigation was made, there was no sign of

intergradation between the two species. The nature of the isolating mechanisms is not known in this case. Season and time of day of swarming seem identical. Evidence for specific distinctness was also found for two European "varieties" (*niger* and *alienus*) of the *Lasius niger* complex (Diver, 1940).

Among the wasps of the genus Polistes, Bequaert (1940) recognized in the species *fuscatus* 18 "varieties" which differ in color, but not in structure. Near Kirkwood, Missouri, Rau (1942, 1946) finds three of these "varieties," *pallipes*, *variatus*, and *rubiginosus*, but they behave like three good species. Each of the three is clearly characterized by distinctive color patterns and intermediates are absent. The color characters are correlated with seven distinct behavior or ecological differences. The naturalist Rau finally exclaims: "I, for my part, am only too happy to meet and greet *pallipes* and *variatus* as two distinct species, but after all, I am not a taxonomist." The two Californian "varieties" of *Polistes fuscatus*, recognized by Bequaert, differ not only in color, habits and ecology, but also in having different strepsipteran parasites; the parasites are frequent in one, rare in the other (Bohart, 1942). To apply the term variety to such obvious species is possible only for adherents of an extremely rigid morphological species concept.

The order of Diptera is another group of insects that is rich in sibling species. McCarthy (1945) found that specimens identified by a Sciara specialist as belonging to *Sciara fenestralis* actually belonged to two exceedingly similar species. The two species failed to produce hybrids and the banding patterns of the salivary chromosomes did not match. Females of species *A* produce regularly both male and female offspring with the males in excess (bisexual reproducer) while a given female of species *B* produces exclusively offspring of one sex, male or female (unisexual producer). Morphological differences were negligible. A similar case, involving *Sciara ocellaris-reynoldsi* had previously been described.

The number of known sibling species in the genus Anopheles is rapidly increasing, although there is still a tendency among the medical entomologists to label such non-interbreeding natural populations as varieties. This is evident in Aitken's revision (1945) of the western North American species and other recent works. On the other hand, more and more authors follow Bates' logical and consistent step (Mayr, 1942) to raise such "varieties" to the rank of full species. This was done for *Anopheles melas*, a sister species of *A. gambiae* (Ribbands, 1944).

It is fortunate for the study of sibling species that they are very common in the genus Drosophila. There is every intergradation between pairs of species which in spite of their similarity can be distinguished by the specialist, like *Drosophila melanogaster* and *D. simulans*, and pairs like

Drosophila willistoni and *D. equinoxialis* (Dobzhansky, 1946), that are morphologically entirely indistinguishable, except for a slight average size difference. Flies of *D. willistoni* reach sexual maturity more quickly, since at the age of 24 hours after emergence from the pupae half of the females contain sperm, while in *D. equinoxialis* this proportion of inseminated females is reached at 48 hours. Cross matings are completely sterile and not a single hybrid was produced. The widespread occurrence of morphologically very similar species of Drosophila has been clearly established, chiefly through the work of Patterson and his co-workers. Such sibling species are *Drosophila macrospina* and *D. subfunebris* (Mainland, 1942), the *Drosophila virilis* group, consisting of *D. virilis, americana, texana, nova-mexicana, montana,* and *lacicola* (Patterson, Stone and Griffen, 1942; Patterson and Griffen, 1944), the *mulleri* group, consisting of *mulleri, aldrichi, mojavensis, arizonensis,* and *buzzattii* (Crow, 1942), the *cardini* group (Streisinger, 1946), and *Drosophila pseudoobscura* and *D. persimilis* (Dobzhansky and Epling, 1944). Additional sibling species complexes from Europe (*obscura* complex) and South America have been mentioned in preliminary publications. The significance of these findings will be discussed at the end of this section.

Only few sibling species have been described among beetles. In the rice weevil, *Calandra oryzae,* two strains have been described by Zumpt, Richards, and Birch (1946) which differ in size and various physiological characters, but not morphologically. A few F_1 hybrids are produced in single choice crosses. F_1 females lay eggs and are inseminated by males of the large strain, but all eggs laid by such females are unfertilized. The indication is that sibling species are involved. Two borers of the genus Cyllene are also very similar, but have different hosts and breeding seasons (King, 1943).

Sibling species are common among moths, although careful studies of the genital armatures have shown that the differences between the species are often more pronounced than is apparent from the external morphology. Most of the common food-pest moths have turned out to be groups of sibling species (Corbet, 1943). Of the four species of the *Tinaea granella* complex, only two have been found to infest grain, and of these *T. granella* prefers wheat, *T. infimella* rye. As in mosquitoes, the larvae of these moths are sometimes more distinct than the adults.

Many of the species of insects that attack forest trees also seem to be really groups of sibling species. Brown and Mackay (1943) show that the budworm (*Cacoelia* [*Archips*] *fumiferana,* Tortricidae) which lives on balsam fir and spruce is different from the one on jack pine, although there is no clear-cut morphological distinction. Either species may occasionally infest the "wrong" host without any change of characters.

The work of Cantrall (1943) has shed much light on the so-called biological races of tree-crickets of the *Nemobius fasciatus* group. The three species *N. fasciatus*, *N. socius*, and *N. tinnulus* occur together in the George Reserve in Michigan. In captivity any species will mate with any other, but only the *fasciatus* x *tinnulus* cross is fertile. Both species have very distinctive songs and F_1 hybrids have an intermediate song, which is not heard in nature. In addition to the song differences there are very definite differences in habitat preference. *N. fasciatus* lives in dry grasslands, *N. socius* in marshes, and *N. tinnulus* in sunny oak-hickory forests. Some entomologists still refer to these species as "physiological races."

It is impossible to make statements concerning presence and frequency of sibling species in groups that are not well-known taxonomically. This is true not only for most families of insects, but also for molluscs (*e.g.* genus Pisidium) and most internal parasites. Among marine organisms also only few sibling species complexes have been studied. A study of the *Gammarus pulex* complex (crustaceans) (Sexton, 1942) is a notable exception. Others occur in limpets, *e.g.* in the genera Patella (Eslick, 1940) and Acmaea (Test, 1946).

What corresponds to sibling species in the higher animals exists also among the protozoa. An earlier summary of the work was published by Kimball (1943), but there is a more detailed study by Sonneborn and Dippell (1946) on the situation in *Paramecium aurelia*. The seven known so-called "varieties" of *P. aurelia* belong to two groups, *A* and *B*. No variety of group *A* reacts sexually with a variety of group *B*, nor any variety in group *B* with any other variety. The four varieties of group *A* do not have complete sexual isolation, as believed earlier, but there are potent bars to the exchange of genes. Sexual isolation in nature may well be complete. Although these "varieties" in Paramecium have all the biological characteristics of good species, it would serve no good end to give them distinct species names until the analysis has been carried a good deal further.

Morphologically indistinguishable so-called biological races occur commonly among pathogenic protozoans, particularly in Plasmodium and Trypanosoma (Hoare, 1943). However, it seems to be unknown whether they are reproductively isolated and the question cannot yet be answered whether or not they correspond to the sibling species of higher animals.

The Evolutionary Significance of Sibling Species. This review shows that it has been clearly established during recent years that morphologically similar or indistinguishable species are not an exception but seem to occur in all well-studied groups of animals, although with greater frequency in some orders and families. Furthermore, that there is no principal difference between sibling species and strikingly distinct congeneric species. In fact,

there is a complete gradation between the cases in which congeneric species are conspicuously different, only slightly different, and completely identical in external morphology. However, even these latter sibling species are reproductively isolated from each other and there is no evidence of a transfer of genes from one species to the next. In short, they satisfy all the requirements of a biological species definition.

It has been asked how the evolution of sibling species can be explained (Huxley, 1943). Two explanations seem feasible. One is that they are species *in statu nascendi* and that they are of such recent origin that they have had no time yet to acquire significant morphological differences. A corollary of this hypothesis would be to assume that reproductive isolation had been acquired through one or only a few steps, and that sibling species are on the whole genetically nearly identical. All the known facts, particularly those derived from a study of *Drosophila pseudoobscura-persimilis*, and *Anopheles maculipennis* complex, and the *Paramecium aurelia* varieties, indicate that this assumption is incorrect. Nearly every physiological and ecological character of these sibling species, that has been carefully studied, has been found to be genetically distinct in the various sibling species. Even though I do not agree with Epling's hypothesis (Epling, 1944) of possible early Tertiary origin of the species *Drosophila pseudoobscura* and *D. persimilis*, the genetic differences between them, and their failure to hybridize in nature where their ranges overlap indicate clearly that they are *not* species *in statu nascendi*.

The alternative explanation would be that the morphological characters of sibling species are genetically so well integrated that they are not visibly affected by the mutational steps that have produced ecological and reproductive isolating mechanisms. If such were the case it might be interpreted as indicating that in certain genera (*e.g.* Drosophila, Anopheles) a certain standardized morphology has a definite selective value which prevents visible changes. What is particularly puzzling is the fact that those sibling species that have been tested genetically like *Drosophila pseudoobscura* and *D. persimilis* seem to have normal mutation rates and the expected share of visually conspicuous mutations. The problem of the similarity of sibling species seems akin to that of phylogenetically conservative characters and of the characters of the higher categories (Mayr, 1942). The taxonomist cannot do much about the solution of these problems except to call them to the attention of the geneticist.

2. *Physiological Races*

Many sibling species were described originally as physiological races. Adherents of a morphological species concept define as physiological or

biological races "morphologically indistinguishable populations which occur in different ecological niches at the same locality." Reviews of such cases were given by Thorpe (1930, 1940) although he includes in his reviews a rather heterogeneous medley of cases as pointed out by Mayr (1942). As more and more so-called physiological races are being unmasked as sibling species, the question arises whether genuine physiological races exist at all. This question remains to be answered. Not a single bona fide physiological race has come to my attention.

Indeed, the evidence in favor of the existence of physiological races is rather weak. The term itself has often been applied vaguely or incorrectly as, for example, to morphologically indistinguishable geographical races (Thomson, 1943a and b). It is, for instance, not justified to call the Starling (*Sturnus vulgaris*) of Great Britain a physiological race, even though the differences between the British and northern European populations are restricted to differences in the breeding cycle and do not affect plumage characters or size. The British Starling is as typically a *geographical* race as are morphologically distinguishable geographical races. Other so-called physiological races are at least microgeographical races. To what confusion the failure of a proper definition can lead is demonstrated in an analysis of so-called biological races in the pea aphid (*Macrosiphum pisi*). Harrington (1945) isolated individual aphid females and tested the virulence to pea plants of their parthenogenetically produced offspring. He found that some of these lines were of weak, some of strong, and some of very strong virulence. The data indicate a certain amount of geographical variation, but an even greater amount of intrapopulation variability in virulence. This is indicated by the existence of differences in virulence among lines originating from the same locality (*e.g.* Blacksburg, Virginia, 1941 and 1942, or Madison, Wisconsin, W 41-1 and W 41-2). However, no evidence is presented that the individuals from which the various parthenogenetic lines were established belonged to different races. The application of the term biological races to these temporary clones is misleading.

The only cases where physiological races might conceivably occur are among parasites and oligophagous plant feeders. Such physiological races might coexist at a single locality, restricted to different hosts. But even for them the available evidence is contradictory. It now appears that the concept "physiological race" might well be a logical impossibility. How can ecologically distinct, wholly sympatric populations evolve within a species unless gene flow between them is prevented by sexual isolation? And if there is sexual isolation, why should these populations not be called species? It is likely that the term "physiological race" will largely disappear from the literature.

An inquiry into the role of non-genetic, conditioned ecological speciation has been made in a separate publication (Mayr, 1947).

3. *Subspecies Versus Allopatric Species*

If the species of a given locality are compared with each other it is found, as was shown above, that they differ in certain attributes which serve as isolating mechanisms. Differences in their ecological requirements, as well as morphological differences, usually can also be demonstrated. These are the three test criteria commonly employed to determine the status of sympatric populations. The most important of these criteria is the presence or absence of isolating mechanisms and this is the very criterion which cannot be used (except experimentally) to decide the status of isolated populations. It is for this reason that classification of allopatric populations is often subject to a considerable amount of disagreement among taxonomists. Many solutions for this dilemma have been proposed but all of them are beset with difficulties.

Some taxonomists insist that all such populations be treated as full species "until it is proven that they are subspecies." Such a solution is, of course, impractical because it is impossible in most of these cases to provide clear-cut proof, one way or the other. Furthermore, this solution overlooks the fact that it is just as serious an error to call a population a species if it is really only a subspecies than to call it a subspecies if it is a species.

The second solution is to treat all populations as full species which are not connected by intergradation. This procedure is founded on the correct observation that populations that are connected by intergradation are conspecific, and jumps from this observation to the reversed conclusion that populations which are not connected by intergradation are not conspecific. This conclusion is correct only so far as sympatric or contiguous populations are concerned because with them the lack of intergradation proves the absence of interbreeding and thus constitutes *de facto* proof of specific distinctness. There is, however, much direct and indirect evidence that it does not necessarily apply to isolated, allopatric populations. Geographical isolation is not an intrinsic isolating mechanism, and there is no guarantee that the morphological hiatus caused by the temporary stop in the gene flow is proof of the evolution of isolating mechanisms.

With direct proof unavailable it becomes necessary to decide such cases by inference. Several kinds of evidence are available. All of these are based on the observation that reproductive isolation is correlated with a certain amount of morphological difference, which is rather typical for any given genus. It thus becomes the task of the taxonomist to use this evidence to work out a yardstick which can be applied to isolated popu-

lations. Three sets of observations on morphological differences supply evidence that can be utilized to calibrate such a scale:

(1) Degree of difference between sympatric species. — This is very slight in certain genera, as in the flycatcher genus Empidonax and the mosquito genus Anopheles, but extraordinarily large among the birds of paradise.

(2) Degree of difference between intergrading subspecies within widespread species. — The striking morphological differences between such forms in some genera show that morphological difference is not necessarily correlated with reproductive isolation.

(3) Degree of difference between hybridizing populations. — Subspecies or groups of subspecies within a species become sometimes temporarily separated from each other through the development of a geographical barrier, but merge again after the breakdown of the barrier. There are many cases on record that prove that free interbreeding was possible after the breakdown even in cases where considerable morphological gaps had developed. The juncos (Junco) and flickers (Colaptes) in North America and the megapodes (Megapodius) and whistlers (Pachycephala) in Oceania are good examples among birds for such free interbreeding of morphologically strongly differentiated populations.

It is preferable for practical as well as for scientific reasons to treat all doubtful allopatric populations as subspecies. The scientific reason is that the mere fact that a population was unable to overlap the range of its nearest relative implies that it has been unable so far to develop isolating mechanisms that would permit coexistence. There is no more formidable zoogeographical barrier for a subspecies than the range of another subspecies. The practical reason is that the use of trinomials conveys at once two important pieces of information: (1) closest relationship and (2) allopatry. Such information is very valuable, particularly in large genera. The treatment of all such allopatric forms as separate species, on the other hand, seems to have not a single practical advantage to say nothing of its scientific weakness. For arguments pro and contra the above expressed views see Schmidt (1944) and Myers (1942), also, Criteria Symposium (Bogert *et al*, 1943).

One of the objections raised against the above-described yardstick method is that it leads to the recognition of subspecies of very different value. It extends the range of the subspecies from the poorly-defined intergrading population to the well-defined, isolated, allopatric form. Those who raise this objection forget that the species concept gains in conciseness of definition what the subspecies concept may lose. The species of the older workers were a heterogeneous medley of allopatric forms (without reproductive isolation) and of true species (sympatric and

allopatric). To restrict the application of species rank to populations that are reproductively isolated gives much more precise limits to the species than was formerly possible.

VI. Conclusion

The observations and discussions of the preceding pages on the nature of species can be summarized as follows. Species are natural units characterized by their reproductive isolation from other such units. The species of the taxonomist, so far as based on morphological criteria, are merely inferences from the species in nature. Whenever there is doubt as to the specific rank of a natural population, the analysis of the morphological criteria will have to be supplemented by a study of ecological differences and of the completeness of reproductive isolation.

VII. References

Aitken, Th. H. G., *Univ. Calif. Publ. Ent.* **7**, 273–364 (1945).
Beauchamp, R., and Ullyott, P., *J. Ecol.* **20**, 200–208 (1932).
Beheim, D., *Zeitschr. Morph. Ökol. Tiere* **39**, 21–46 (1942).
Bequaert, J. C., *J. N. Y. ent. Soc.* **48**, 1–31 (1940).
Birch, L. C., *Austr. J. exp. Biol. med. Sci.* **24**, 123–125 (1946).
Blair, A. P., *Biol. Symposia* **6**, 235–249 (1942).
Blair, W. F., and Howard, W. E., *Contr. Lab. Vertebr. Biol.* **26**, 1–19 (1944).
Bogert, C. M., Blair, W. F., Dunn, E. R., Hall, E. R., Hubbs, C. L., Mayr, E., and Simpson, G. G., *Ann. N. Y. Acad. Sci.* **44**, 105–188 (1943).
Bohart, G. E., *Pan-Pacific Ent.* **18**, 30 (1942).
Brown, A. W. A., and Mackay, M. R., *Canad. Ent.* **75**, 202 (1943).
Cantrall, I. J., *Misc. Publ. Mus. Zoöl. Univ. Mich.* **54**, 3–182 (1943).
Chapin, J. P., *Ibis* **86**, 543–545 (1944).
Corbet, A. S., *Nature* **152**, 742–743 (1943).
Crow, J. F., *Univ. Texas Publ.* **4228**, 53–67 (1942).
Darlington, C. D., *Nature* **152**, 315–319 (1943).
Dice, L. R., *Ecology* **23**, 199–208 (1942).
Diver, C., in "The new systematics," 303–328, Clarendon Press, Oxford (1940).
Jour. Ecol., 33, 115–116 (1945).
Dobzhansky, Th., "Genetics and the origin of species," 2d rev. ed. 446 pp. Columbia University Press, New York (1941).
Publ. Carneg. Instn. **554**, 47–144 (1944a).
Proc. nat. Acad. Sci., Wash. **30**, 335–339 (1944b).
Ecology **27**, 205–211 (1946).
Dobzhansky, Th., and Epling, C., *Publ. Carneg. Instn.* **554**, 1–46 (1944).
Dobzhansky, Th., and Mayr, E., *Proc. nat. Acad. Sci., Wash.* **30**, 238–244 (1944).
Dobzhansky, Th., and Streisinger, G., *Proc. nat. Acad. Sci., Wash.* **30**, 340–345 (1944).
Elton, C., *J. Anim. Ecol.* **15**, 54–68 (1946).
Emerson, A. E., *Amer. Nat.* **77**, 97–118 (1943).
Ent. News **56**, 14–19 (1945).
Epling, C., *Publ. Carneg. Instn.* **554**, 145–183 (1944).
Eslick, E., *Proc. Linn. Soc. Lond.* **152**, 45 (1940).

Ford, E. B., *Biol. Rev.* **20,** 73–88 (1945).
Fox, H. M., *Nature* **156,** 475 (1945).
Nature **157,** 511 (1946).
Gause, G. F., "The struggle for existence." 163 pp. The Williams and Wilkins Co. (1934).
Gibson, N. H. E., *Trans. R. Entom. Soc. London* **95,** 263–294 (1945).
Gordon, M., *Advances in Genetics* **1,** 95–132 (1947).
Gordon, M., and Benzer, P., *Zoologica, N. Y.* **30,** 57–72 (1945).
Gösswald, Karl, *Z. angew. Ent.* **28,** 62–124 (1941).
Gregg, E. V., *Ann. ent. Soc. Amer.* **38,** 529–548 (1945).
Harrington, C. D., *J. econ. Ent.* **38,** 12 (1945).
Hesse, R., Allee, W. C., and Schmidt, K. P., "Ecological animal geography." 597 pp. John Wiley and Sons, New York (1937).
Hoare, C. A., *Biol. Rev.* **18,** 137–144 (1943).
Hobbs, H. H., and Marchand, L. J., *J. Tenn. Acad. Sci.* **18,** 6–35 (1943).
Hovanitz, W., *Caldasia, Bol. Inst. Ci. Nat. Un. Colombia, Bogota* **3,** 283–300 (1945).
Physiol. Zoöl. **19,** 35–53 (1946).
Huxley, J. (Editor) The new systematics, 1–46, Clarendon Press, Oxford (1940).
"Evolution. The modern synthesis," 645 pp. George Allen and Unwin Ltd., London (1942).
Nature **347** (1943).
Irwin, M. R., *Biol. Rev.* **21,** 93–100 (1946).
Irwin, M. R., and Cumley, R. W., *Amer. Nat.* **77,** 211–233 (1943).
Irwin, M. R., and Cole, L. J., *Genetics* **30,** 487–495 (1945).
Isely, F. B., *Ecology* **27,** 128–138 (1946).
Jennings, H. S., and Opitz, P., *Genetics* **29,** 576–583 (1944).
Johnson, D. H., *Univ. Calif. Publ. Zoöl.* **48,** 63–148 (1943).
Kennedy, W. A., *Univ. Toronto Stud. Biol. Ser.,* **51,** 23–66 (1943).
Kimball, R. F., *Quart. Rev. Biol.* **18,** 30–45 (1943).
King, E. W., *Amer. Midl. Nat.* **29,** 180–182 (1943).
Klauber, L. M., *Herpetologica* **1,** 18–27 (1936).
Bull. Zool. Soc. San Diego **15,** 1–23 (1939).
Kullenberg, B., *Zool. Bidrag fran Uppsala* **23,** 367–386 (1946).
Lack, David, *Ibis* 260–286 (1944).
J. Anim. Ecol. **14,** 12–16 (1945).
"Darwin's finches." 208 pp. Cambridge University Press, (1947).
Lamy, R., *Proc. roy. Soc. Edinb.* **62,** 9–19 (1943).
Landsteiner, K., "The specificity of serological reactions." Rev. ed. Harvard University Press, Cambridge, Mass. (1945).
Levene, H., and Dobzhansky, Th., *Proc. nat. Acad. Sci.* **31,** (1945).
Levine, H. P., *Biol. Bull.* **85,** 52–59 (1943).
Lorenz, K., *J. Orn. Suppl.* **3,** 194–294 (1941).
Mainland, G. B., *Univ. Texas Publ.* **4228,** 98 (1942).
Mayr, E., "Systematics and the origin of species." 334 pp. Columbia University Press, New York (1942).
Zoologica, N. Y. **30,** 110 (1945).
Proc. nat. Acad. Sci. **32,** 57–59 (1946a).
Proc. nat. Acad. Sci. **32,** 128–137 (1946b).
Proc. Acad. nat. Sci. Philad. **98,** 271–276 (1946c).
Evolution **1,** 263–288 (1947).
Mayr, E., and Dobzhansky, Th., *Proc. nat. Acad. Sci.* **31,** 75–82 (1945).

Mayr, E., and Dunn, L. C., *Amer. Midl. Nat.* **31,** 88–95 (1944).
McCarthy, M. D., *Amer. Nat.* **79,** 104–121 (1945).
McGibbon, W. H., *Genetics* **29,** 407–419 (1944).
Meise, W., *J. Ornith.* **84,** 631–672 (1936).
Moore, J. A., *Biol. Symp.* **6,** 189–213 (1942a).
Biol. Bull. **83,** 375–388 (1942b).
Bull. Amer. Mus. nat. Hist. **82,** 349–369 (1944).
Proc. nat. Acad. Sci. **32,** 209–212 (1946a).
Genetics **31,** 304–326 (1946b).
Muller, H. J., *Biol. Symp.* **6,** 71–125 (1942).
Myers, G. S., *Occ. Pap. Mus. Zoöl., Univ. Mich.* **460,** 1–13 (1942).
Omori, N., *Acta Jap. Med. Trop.* **1,** 27–154 (1939).
Patterson, J. T., *Biol. Symp.* **6,** 271–287 (1942).
Proc. nat. Acad. Sci. **32,** 202–208 (1946).
Patterson, J. T., and Dobzhansky, Th., *Genetics* **30,** 429–438 (1945).
Patterson, J. T., and Griffen, A. B., *Univ. Texas Publ.* **4445,** 194–211 (1944).
Patterson, J. T., Stone, W. S., Griffen, A. B., *Univ. Texas Publ.* **4228,** 162–183 (1942)
Patterson, J. T., *et al.*, *Biol. Symp.* **6,** 217–287 (1942).
Patterson, J. T., (and others), *Univ. Texas Publ.* **4228,** 7–200 (1942).
Patterson, J. T., (and others), *Univ. Texas Publ.* **4445,** 1–223 (1944).
Pfaler-Collander, E. v., *Acta Zool. Fennica* **30,** 114 pp. (1941).
Pope, C. H., *Zoöl. Ser. of Field Mus.* **24,** 249–252 (1941).
Rau, Phil, *Ann. ent. Soc. Amer.* **35,** 335–338 (1942).
Ann. ent. Soc. Amer. **39,** 11–27 (1946).
Reighard, J., *Papers Mich. Acad. Sci., Arts, and Letters* **28,** 397–423 (1943).
Rendel, J. M., *J. Genetics* **46,** 287–302 (1945).
Ribbands, C. R., *Ann. Trop. Med. Parasit.* **38,** 85–99 (1944).
Schmidt, K. P., *Ecology* **25,** 255 (1944).
Sexton, E. W., *J. Mar. biol. Ass. U. K.* **25,** 575–606 (1942).
Simpson, G. G., *Bull. Amer. Mus. Nat. Hist.* **85,** 1–350 (1945).
Smith, H. M., *Amer. Midl. Nat.* **29,** 245–251 (1943).
Sonneborn, T. M., and Dippell, R. V., *Physiol. Zoöl.* **19,** 1–18 (1946).
Spieth, H., *Evolution* **1,** 17–31 (1947).
Stalker, H. D., *Genetics* **27,** 238–257 (1942).
Streisinger, G., *J. N. Y. Ent. Soc.* **54,** 105–113 (1946).
Stresemann, E., *J. Ornith.* **87,** 299–425 (1939).
Sturtevant, A. H., *J. Anim. Behavior* **5,** 351–366 (1915).
Univ. Texas Publ. **4213,** 5–51 (1942).
Ecology **25,** 476–477 (1944).
Test, A. R., *Ecology* **26,** 395–404 (1945).
Contr. Lab. Vert. Biol., Ann Arbor, Mich. **31,** 1–24 (1946).
Thomson, A. L., *Bull. Brit. Ornith. Club* **63,** 73–80 (1943a).
Bull. Brit. Ornith. Club **64,** 1–15 (1943b).
Thorpe, W. H., *Biol. Rev.* **5,** 177 (1930).
in "The new systematics," 341–364 Clarendon Press, Oxford (1940).
Wagner, R. P., *Univ. Texas Publ.* **4445,** 104–128 (1944).
Wallace, B., and Dobzhansky, Th., *Proc. nat. Acad. Sci.* **32,** 226–234 (1946).
Wharton, L., *Univ. Texas Publ.* **4228,** 23–50 (1942).
White, M. J. D., "Cytology and evolution," 375 pp., Cambridge University Press, Cambridge, 1945; Macmillan Co., New York, 1946.
Wing, L., *Condor* **48,** 154–157 (1946).

The Cytology and Genetics of the Wheats and their Relatives

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CONTENTS

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I. Introduction

Because of the economic importance of wheat, the genus Triticum has long been of interest to geneticists. Since the common species of wheat are polyploid, however, only limited genetic progress has been made. Numerous characters have proved dependent on duplicate or triplicate factors, and many others have defied precise analysis.

Cytological study of the wheats has been more rewarding. Triticum serves as the classical example of an allopolyploid series. There are three groups of species, with chromosome numbers of $n = 7$, 14, and 21. With few exceptions, the species within each group have homologous chromosomes; and each of the two polyploid groups apparently has the chromosomes of the group below it, plus seven additional pairs non-homologous with any of those already present. Hybrids of Triticum species are readily made with certain members of the other genera of the sub-tribe Triticinae — namely, Aegilops, Agropyron, Secale, and Haynaldia — and close relationships are indicated with certain species of Aegilops and possibly Agropyron.

Correlated cytological and genetical studies of the polyploid wheats have largely been confined to the so-called pentaploid hybrids between hexaploid ($n = 21$) and tetraploid ($n = 14$) wheats. Most of the remainder of the cytogenetic work has been done with deficiencies and duplications of whole chromosomes in the hexaploid *T. vulgare* (common wheat) — work largely limited until recently to deficiency for a particular chromosome, the "speltoid" chromosome.

II. Relationships in the Triticinae

Perhaps in no other group has the concept of genome individuality been carried so far as in the Triticinae.

Every species in the five genera of the Triticinae has either seven pairs of chromosomes or some multiple of seven pairs. From this fact and from cytological data from hybrids, it has been assumed that the chromosome complement of each polyploid species consists of groups (or genomes) of seven pairs of chromosomes each. Each of these genomes is considered an entity, homologous or homoeologous with the chromosomes of a particular diploid species. Relationships are indicated by giving each genome a symbol, as Kihara and Lilienfeld (1932) and Kihara (1940a, 1945), especially, have done.

Assessment of the degree of relationship of species belonging to the same or different genera has been based largely on the amount and kind of chromosome pairing found at meiosis in their F_1 hybrids. This method is open to some criticism because of the possibility of non-homologous association, such as found by Levan (1942) in haploid rye. Apparently,

however, few such associations persist until metaphase, where observations on pairing in hybrids are normally made.

The concept of relationship by genomes tends to overlook the fact that in polyploid species translocations may occur between chromosomes belonging to different genomes, with a consequent tendency for loss of individuality of the genomes. Identification of the parent species of a long-established allopolyploid may thus be impossible from cytological data alone. Also, the occurrence of somewhere near seven chromosome pairs in hybrids between two polyploid species need not signify their possession of a genome in common, since the pairs may involve chromosomes from more than one genome in each species. Where good pairing involving all the chromosomes of one or both parents is observed, however, there seems to be sufficient reason for postulating relationship. Where pairing is incomplete, caution must be exercised (Love, 1941), and other criteria of relationship, particularly morphology and hybrid fertility, considered carefully. The genome designations tend to be oversimplifications, but they probably come close to the true picture in the majority of cases, and near enough in most others to serve as useful guides to further research.

Pathak (1940) and Camara (1943) hold that the diploid species of wheat are actually aneuploids, having been derived from a type with five pairs of chromosomes. Even should this hypothesis be accepted, there can be little doubt that the existing genera of the Triticinae all had a common ancestor with seven pairs of chromosomes.

The cytology of hybrids in the Triticinae was reviewed by Aase in 1935, and again in 1946. Kihara (1937a) compiled in tabular form all the cytological data then available on hybrids in this group.

A. *Genome Homologies in Triticum*

The known species of wheat are listed in Table 1. In addition to the species given, there are several of doubtful validity. *T. thaoudar* Reut. is sometimes given specific rank, but is here considered a variety of *T. aegilopoides*. (*T. aegilopoides* itself is often included in *T. monococcum*.) *T. pyramidale* (Del.) Perc. and "*T. orientale* Perc." are frequently separated from *T. durum*, with which they are here included. "*T. speltiforme* Vav." and "*T. rigidum* Vav." are sometimes split off from *T. vulgare*.

The genomic formulae given in Table 1 are those used by Kihara (1924) and Lilienfeld and Kihara (1934). The correspondence of genome *A* of the *AB* tetraploids with *A* of the diploids is by no means perfect, as Kihara and Lilienfeld (1932) have pointed out, but there seems little doubt that the former is derived from the latter; that is, that the tetraploid group arose as an amphidiploid hybrid of *T. monococcum* or *T. aegilopoides*

and some other species with $n = 7$. The other species has not yet been identified; and, indeed, positive identification may never be possible, because of the many changes that must have occurred in the *B* genome since its incorporation into the allotetraploid.

TABLE 1

The Species of Triticum and Their Genomic Formulae

Species	*Chromosome number* (*n*)	*Formula*
T. aegilopoides (Link) Bal.	7	*A*
T. monococcum L.	7	*A*
T. dicoccoides Körn.	14	*AB*
T. dicoccum Schrank	14	*AB*
T. turgidum L.	14	*AB*
T. persicum (Boiss.) Aitch. and Hemsl.	14	*AB*
T. polonicum L.	14	*AB*
T. durum Desf.	14	*AB*
T. timopheevi Zhuk.	14	*AG*
T. armeniacum (Jakubz.) Makush.	14	*AG*
T. spelta L.	21	*ABD*
T. vulgare Vill.	21	*ABD*
T. macha Dekap. and Menab.	21	*ABD*
T. vavilovi (Tuman.) Jakubz.	21	*ABD*
T. compactum Host	21	*ABD*
T. sphaerococcum Perc.	21	*ABD*

Concerning the close relationship of the two emmer genomes with the *A* and *B* of the hexaploids, there is general agreement. Love (1941) has emphasized that hybrids of certain varieties of emmers and vulgares have striking irregularities in chromosome pairing; but it is true that others of the so-called pentaploid hybrids have fourteen "good" meiotic pairs quite regularly. The *G* of *T. timopheevi* and *T. armeniacum* has been designated *β* by Kostoff (1936a), but Lilienfeld and Kihara (1934) and Kihara (1937a) believe that the homology with the *B* of emmers is slight. As Love (1941) points out, it is probable that the *A* of *T. timopheevi* (and *T. armeniacum*) is also differentiated from the *A* of the other tetraploid wheats; but a common origin of the *A*'s from a diploid wheat seems likely, whereas *B* and *G* are probably derived from different species.

The letter *D* is used to represent the third genome of the hexaploid wheats. American and British workers commonly use *C* instead of *D*;

but *D* was proposed by Kihara in 1924, while *C* was not used until 1926 (Gaines and Aase, 1926). Furthermore, most of the subsequent work of identifying and symbolizing the genomes of the relatives of wheat has been done by Kihara and his co-workers, who have based their designations on the use of *D* rather than *C*.

In general, species of Triticum with the same genomic formula form fertile hybrids with regular meiotic pairing. (*T. timopheevi* × *T. armeniacum* has good pairing but no fertility, according to Svetozarova, 1939.) Hybrids between groups have more or less regular pairing between the chromosomes of corresponding genomes, although only in *ABD* × *AB* does the pairing actually simulate that in the parent species. Only the latter intergroup hybrids have appreciable fertility.

After much speculation and research by many investigators (reviewed by McFadden and Sears, 1946) as to the origin of the *D* genome of the hexaploid wheats, this chromosome set has finally been identified as homologous with the genome of *Aegilops squarrosa* (Kihara, 1944; McFadden and Sears, 1944, 1946). An allopolyploid of *T. dicoccoides* × *Ae. squarrosa* has been produced (McFadden and Sears, 1944, 1946), and this forms fertile, meiotically regular hybrids with *T. vulgare* and *T. spelta*. The allopolyploid itself resembles *T. spelta* very closely, leading to the hypothesis that *T. spelta* arose as an allopolyploid of either *T. dicoccoides* or *T. dicoccum* × *Ae. squarrosa*. *T. vulgare* is assumed to be a segregate of a cross of *T. spelta* with the now extinct Lake Dweller wheat, which is believed to have been a tetraploid.

The origin of the tetraploid wheats is more obscure, because a much longer time has elapsed since these wheats arose. McFadden and Sears (1946) have suggested that the Lake Dweller wheat may have been an amphidiploid of *T. monococcum* × *Agropyron triticeum*. *T. persicum* is considered by these authors to be a derivative of the Lake Dweller wheat. Camara (1935) believes that the tetraploid wheats may have arisen from the diploids through autotetraploidy.

B. *Genome Homologies in Aegilops*

The genus Aegilops is of interest here chiefly because it has provided the *D* and possibly the *B* genome of common wheat. The known species are listed in Table 2, together with the genomic formulae given them by Kihara (1940a and b; 1945).

In Kihara's formulae, for which the full supporting data have not yet been presented, the use of the symbol C^u for the genome of *Ae. umbellulata* and its derivatives seems to overemphasize the closeness of relationship to the genome of *Ae. caudata*. Actually, the data — both cytological and morphological — show no closer relationship of *Ae. caudata* to *Ae. umbel-*

lulata than to *Ae. comosa,* to which Kihara gives a different letter, *M*. In at least one tetraploid member of the Pleionathera, *Ae. triuncialis,* the *umbellulata* genome (and the *caudata* genome, too) is considerably modified (Sears, 1941), but this is not indicated by Kihara's designations.

TABLE 2

The Species of Aegilops and Their Genomic Formulae

Section	*Species*	*Chromosome number (n)*	*Formula*
Anathera	*Ae. mutica* Boiss.	7	Mt
Platystachys	*Ae. bicornis* (Forsk.) Jaub. et Spach	7	S^b
	Ae. sharonensis Eig	7	
	Ae. longissima Schw. et Muschl.	7	S^l
	Ae. speltoides Tausch	7	S
Pachystachys	*Ae. squarrosa* L.	7	D
	Ae. ventricosa Tausch	14	DM^v
	Ae. crassa Boiss.	14	DJ
	Ae. crassa Boiss.	21	$DJ + ?$
	Ae. juvenalis (Thell.) Eig	21	
Monoleptathera	*Ae. cylindrica* Host	14	CD
Macrathera	*Ae. caudata* L.	7	C
	Ae. comosa Sibth. et Sm.	7	M
	Ae. uniaristata Vis.	7	M^u
Pleionathera	*Ae. umbellulata* Zhuk.	7	C^u
	Ae. triuncialis L.	14	C^uC
	Ae. variabilis Eig	14	C^uS^v
	Ae. columnaris Zhuk.	14	C^uM^c
	Ae. biuncialis Vis.	14	C^uM^b
	Ae. ovata L.	14	C^uM^o
	Ae. triaristata Willd.	14	C^uM^t
	Ae. triaristata Willd.	21	$C^uM^t + ?$

The two species without formulae have not yet been tested in all the critical crosses. *Ae. sharonensis* is taxonomically very close to *Ae. longissima* (Eig, 1929), and has been found cytologically to have a modified *S* genome.

Two tetraploid species have been synthesized artificially. These are *Ae. cylindrica* from *Ae. caudata* × *Ae. squarrosa* (McFadden and Sears,

1944, 1946) and *Ae. triuncialis* from *Ae. caudata* × *Ae. umbellulata* (Sears, 1941). The former amphidiploid approaches *Ae. cylindrica* fairly closely in chromosome homology, but *Ae. caudata* × *Ae. umbellulata* differs considerably from *Ae. triuncialis* in chromosome constitution.

C. *Genome Homologies in Agropyron*

A detailed consideration of all of the large number of species of Agropyron (nearly 100, according to Tzitzin, 1940) is outside the province of this review. As in the other genera of the Triticinae, the species form a polyploid series starting with $n = 7$; but the degree of polyploidy attained is higher here, extending to $n = 35$. Autopolyploidy is apparently rather frequent also.

About 30 different species of Agropyron have been used in crosses with wheats (see D. C. Smith, 1942, for detailed review). Most of the successful crosses have been made with the species *A. elongatum* (Host) Beauv. ($n = 35$) and *A. intermedium* (Host) Beauv. (*A. glaucum* Desf.) ($n = 21$), which Tzitzin (1933) and many subsequent Russian, Canadian, and American workers have crossed with both hexaploid and tetraploid wheats. Tzitzin (1933) also reported hybrids of these two species with *T. monococcum*. *A. obtusiusculum* Lange ($n = 21$), used successfully in crosses by Kovaleva (1941), is thought by Smith (1942) to belong to the *intermedium* complex. *A. trichophorum* (Link) Richt. ($n = 21$) and *A. junceum* (L.) Beauv. ($n = 14$) have been crossed successfully to tetraploid and hexaploid wheats by Tzitzin (1933), Armstrong (1945) and other workers. Veruschkine (1936a) hybridized wheat with *A. elongatiforme* Drob. and (1936b) reported crosses of *A. cristatum* (L.) Gaertn. with *T. timopheevi*. Tzitzin (1936) found forms of *A. repens* which crossed readily with wheat.

Of all the wheat-Agropyron hybrids produced, only *T. vulgare* × *A. elongatum* consistently sets selfed seeds. Tzitzin (1936) obtained hybrids of *T. vulgare* × *A. intermedium* which set some seed on selfing, and Khijnyak (1937) got selfed seed from several tetraploid wheats × *A. intermedium;* but other investigators have found only indehiscent anthers in these F_1's. Most of the hybrids yield a few seeds when backcrossed to one or the other parent. Several Agropyron characters have reportedly been transferred to wheat-like segregates by Russian investigators, particularly Tzitzin (1940) and his co-workers.

Hybrids of wheat with Agropyron have shown considerable chromosome pairing, particularly where *A. elongatum* was involved. Vakar (1935, 1936, 1937), who reported up to 28 bivalents in *T. vulgare* × *A. elongatum*, postulated that *A. elongatum* has three genomes homoeologous to the three of *T. vulgare*, plus two other genomes homoeologous with each other. He represented these genomes as $A_1B_1D_1X_1X_2$. Peto (1936) questions some

of Vakar's observations, and himself finds only about 21 pairs and 14 univalents in *T. vulgare* × *A. elongatum*, and essentially the same number of associations in *T. dicoccum* × *A. elongatum*. Furthermore, *A. elongatum* itself has the cytological appearance of being largely autopolyploid. Peto therefore suggests that *A. elongatum* is *AXXYY* (or *BXXYY*), with only one genome homoeologous with any in wheat. It is possible that diversity of material may account for some of the differences between the findings of Peto and Vakar (Peto, 1939, has subsequently found fewer than seven univalents in certain plants of *T. vulgare* × *A. elongatum*); but at present Peto's theory is more acceptable, since it takes into consideration the meiotic behavior of *A. elongatum* itself.

A. intermedium (*A. glaucum*) was believed by Vakar (1936, 1937) to have the genomic constitution $A_aD_aX_1$, with A_a and D_a homoeologous with *A* and *D* of *T. vulgare*. He reported up to 14 bivalents in hybrids with *T. vulgare*. Peto (1936) again questions the occurrence of so much pairing, his own preparations showing an average of only about seven pairs. He suggests that *A. intermedium* is *AXY*, and believes that *A. elongatum* may have arisen through chromosome doubling in a hybrid of *A. intermedium* and some tetraploid Agropyron species with the constitution *XY*. Sapehin (1935) found even less pairing in *T. vulgare* × *A. intermedium* than did Peto. Sipkov (1936) found both extremes of pairing, depending on the strain of *A. intermedium* used.

Love and Suneson (1945) found an average of about seven pairs in hybrids of *A. trichophorum* with tetraploid and hexaploid wheats. This suggests that also in this species one genome may be homoeologous to the *A* or *B* of the wheats. No good evidence exists, however, that the homoeology observed here (or in *A. elongatum* and *A. intermedium* × wheats, for that matter) does not involve chromosomes from both the *A* and *B* genomes.

Up to seven bivalents (average 4.8) were found (Östergren, 1940) in the hybrid *T. turgidum* × *A. junceum*, but these were believed due, in part at least, to autosyndesis of *A. junceum* chromosomes. Quadrivalents were observed in *A. junceum* itself.

From the foregoing it is clear that a few species of Agropyron are fairly closely related to Triticum, while others, which do not hybridize with wheat, are presumably not closely related. Nevsky (1936) and especially Veruschkine (1936b) suggest splitting the genus to conform with, among other things, crossability of the various species with wheat; but this suggestion has apparently not met with general approval. There is no direct evidence that any species of Agropyron has been involved in the synthesis of wheat species; but McFadden and Sears (1946), as mentioned previously, suggest that the free-threshing characteristic of the hexaploid

wheats may have been derived from *A. triticeum,* following incorporation of this species into an allotetraploid hybrid.

In view of the close relationship of the genera Aegilops and Triticum, it is not surprising that certain Agropyron species which cross with the wheats should hybridize readily with Aegilops species. Veruschkine (1935) crossed *A. intermedium* with *Ae. juvenalis* (*Ae. turcomanica*), *Ae. speltoides, Ae. crassa, Ae. triaristata, Ae. triuncialis,* and *Ae. variabilis.* These hybrids were reported to be partially fertile. Favorsky (1936) found cytologically that *Ae. speltoides* × *A. intermedium* has 7 bivalents at meiosis, and that *Ae. turcomanica* × *A. intermedium* has 14 to 21 pairs. Evidently Agropyron is at least as closely related to Aegilops as to Triticum.

D. *Genome Homologies in Secale*

Only five species of Secale are commonly recognized (Schiemann, 1932), *S. cereale* L., *S. montanum* Guss., *S. fragile* Bieberst., *S. africanum* Stapf, and *S. vavilovi* Grossh. (Another species, *S. daralgesi,* has recently been proposed by Tumanien, 1938.) These are evidently closely related, all with $n = 7$, and have homologous chromosomes (Kostoff, 1937; Longley and Sando, 1930). Only *S. cereale,* the common cultivated rye, and *S. montanum,* a wild, perennial species, are widely known. Most of the intergeneric crossing work has been done with *S. cereale.*

There is no good evidence that the rye genome is closely related to any other in the Triticinae. Cytological studies of hybrids with members of each other genus (see Kihara, 1937a, for review) have shown no chromosome association that could not be accounted for by autosyndesis of the non-rye chromosomes. Favorsky (1935) found six or seven pairs in a hybrid of *S. cereale* × *A. cristatum* ($n = 14$), but it is now known that the $n = 14$ form of *A. cristatum* is autotetraploid. Ljubimova (1937) reports 3–4 pairs in *S. cereale* × *A. glaucum.*

E. *Genome Homologies in Haynaldia*

Only one species of Haynaldia has been used extensively in hybridization work, *H. villosa* Schur ($n = 7$). One other species, *H. hordeacea* Hack. ($n = 14$), has been described, and reportedly crossed with *Secale cereale* (Zhukovsky, 1944). This genus is obviously closely related to Triticum, Agropyron, and Aegilops, and has, indeed, been included at various times in each of these genera. There is little indication, however, of much homology of *H. villosa* chromosomes with those of any species of the other genera of the Triticinae. Hybrids of *H. villosa* with various species of Triticum and Aegilops have shown little or no close pairing of chromosomes (see Kihara, 1937a, for review), and Kostoff and Arutiunova

TABLE 3

Amphiploids in the Triticinae

Combination	*n*	*Amphiploid produced by*
T. monococcum × *Ae. uniaristata*	7 + 7	Sears, 1939b
Ae. speltoides × *T. monococcum*	7 + 7	Sears, 1941
Ae. bicornis × *T. monococcum*	7 + 7	Sears, unpub.
T. aegilopoides × *Ae. squarrosa*	7 + 7	Sears, 1941
T. aegilopoides × *Ae. umbellulata*	7 + 7	Sears, 1941
T. aegilopoides × *Ae. uniaristata*	7 + 7	Sears, 1941
T. aegilopoides × *H. villosa*	7 + 7	Sears, unpub.
Ae. speltoides × *Ae. umbellulata*	7 + 7	Kihara, 1937b
Ae. speltoides × *Ae. sharonensis*	7 + 7	Sears, 1941
Ae. speltoides × *Ae. uniaristata*	7 + 7	Sears, 1941
Ae. caudata × *Ae. umbellulata*	7 + 7	Sears, 1939b
Ae. caudata × *Ae. speltoides*	7 + 7	Sears, 1941
Ae. caudata × *Ae. squarrosa*	7 + 7	Sears, 1941
Ae. caudata × *Ae. uniaristata*	7 + 7	Sears, 1941
Ae. comosa × *Ae. uniaristata*	7 + 7	Sears, 1941
Ae. sharonensis × *Ae. caudata*	7 + 7	Sears, 1941
Ae. sharonensis × *Ae. umbellulata*	7 + 7	Sears, 1941
Ae. sharonensis × *Ae. uniaristata*	7 + 7	Sears, 1941
Ae. umbellulata × *Ae. uniaristata*	7 + 7	Sears, 1941
Ae. umbellulata × *H. villosa*	7 + 7	Sears, 1941
Ae. bicornis × *H. villosa*	7 + 7	Sears, unpub.
Ae. bicornis × *Ae. squarrosa*	7 + 7	Sears, unpub.
Ae. caudata × *T. dicoccum*	7 + 14	Oehler, 1934
T. dicoccoides × *Ae. caudata*	14 + 7	McFadden and Sears, 1946
Ae. longissima × *T. polonicum*	7 + 14	Sando, 1935
Ae. longissima × *T. durum*	7 + 14	Sorokina, 1937
T. turgidum × *Ae. speltoides*	14 + 7	Britten and Thompson, 1941
T. dicoccoides × *Ae. speltoides*	14 + 7	McFadden and Sears, 1946
T. dicoccoides × *Ae. squarrosa*	14 + 7	McFadden and Sears, 1946
T. dicoccoides × *Ae. umbellulata*	14 + 7	McFadden and Sears, 1947
T. dicoccoides × *Ae. comosa*	14 + 7	McFadden and Sears, 1947
T. dicoccoides × *Ae. sharonensis*	14 + 7	McFadden and Sears, 1947
T. persicum × *Ae. sharonensis*	14 + 7	McFadden and Sears, 1947
T. dicoccum × *Ae. sharonensis*	14 + 7	McFadden and Sears, 1947
T. dicoccoides × *Ae. uniaristata*	14 + 7	McFadden and Sears, 1947
T. turgidum × *H. villosa*	14 + 7	Tschermak, 1930
T. dicoccum × *H. villosa*	14 + 7	Kostoff and Arutiunova, 1937
T. dicoccoides × *H. villosa*	14 + 7	McFadden and Sears, 1947
T. durum × *Secale montanum*	14 + 7	Derzhavin, 1938
T. timopheevi × *Ae. umbellulata*	14 + 7	McFadden and Sears, 1947
T. timopheevi × *Ae. caudata*	14 + 7	McFadden and Sears, 1947

TABLE 3 (*Continued*)

Combination	*n*	*Amphiploid produced by*
T. timopheevi × *Ae. speltoides*	14 + 7	McFadden and Sears, 1947
T. timopheevi × *Ae. bicornis*	14 + 7	McFadden and Sears, 1947
T. timopheevi × *Ae. uniaristata*	14 + 7	McFadden and Sears, 1947
T. timopheevi × *Ae. squarrosa*	14 + 7	McFadden and Sears, 1947
T. durum × *T. monococcum*	14 + 7	Zhebrak, 1939, 1940b
T. monococcum × *T. persicum*	7 + 14	Kasparyan, 1940
T. timopheevi × *T. monococcum*	14 + 7	Kostoff, 1936b
T. vulgare × *S. cereale*	21 + 7	Rimpau (Lindschau and Oehler, 1935) Müntzing, 1939
Ae. ovata × *T. dicoccoides*	14 + 14	Tschermak and Bleier, 1926
Ae. ovata × *T. durum*	14 + 14	Tschermak and Bleier, 1926
Ae. ovata × *T. turgidum*	14 + 14	Tschermak, 1929
Ae. ovata × *T. dicoccum*	14 + 14	Tschermak, 1929
Ae. triuncialis × *T. dicoccum*	14 + 14	Oehler, 1934
Ae. triuncialis × *T. durum*	14 + 14	Oehler, 1936
Ae. cylindrica × *T. durum*	14 + 14	Sears, 1944c
Ae. ventricosa × *T. durum*	14 + 14	Sears, 1944c
T. durum × *T. timopheevi*	14 + 14	Zhebrak, 1939
T. periscum × *T. timopheevi*	14 + 14	Zhebrak, 1941a
T. turgidum × *T. timopheevi*	14 + 14	Zhebrak, 1941b
T. polonicum × *T. timopheevi*	14 + 14	Zhebrak, 1944a
T. timopheevi × *T. vulgare*	14 + 21	Zhebrak, 1943
T. durum × *T. vulgare*	14 + 21	Zhebrak, 1944b
T. durum × *Agr. intermedium* (*glaucum*)	14 + 21	Khijnyak (Hizniak), 1937
T. dicoccum × *Agr. intermedium*	14 + 21	Khijnyak (Hizniak), 1937
T. turgidum × *Agr. intermedium*	14 + 21	Khijnyak (Hizniak), 1937
T. polonicum × *Agr. intermedium*	14 + 21	Khijnyak (Hizniak), 1937
T. persicum × *Agr. intermedium*	14 + 21	Khijnyak (Hizniak), 1937
T. vulgare × *Agr. intermedium*	21 + 21	Peto, 1938

(1937) have demonstrated the lack of homology between the chromosomes of *H. villosa* and *S. cereale*. White (1940) crossed *H. villosa* × *A. glaucum*, but provided no data on chromosome pairing.

III. Amphiploids and Related Types

It is common practice to apply the term amphidiploid to any hybrid whose chromosome number has been doubled. This practice leads to

confusion when one or both of the species going into the hybrid are themselves of polyploid origin, as is frequently true of hybrids in the wheat group. Of use, therefore, is the term amphiploid (Clausen *et al*, 1945), which implies nothing concerning the polyploid or non-polyploid nature of the species involved. This term merely signifies that the chromosomes of two species have been added together, and the "species" thus added may themselves be synthetic, or experimentally produced, so long as they are amphiploids and not autoploids.

In Table 3 is given a list of all the known amphiploids in the Triticinae. For some which have arisen more than once, such as the *T. vulgare* × *S. cereale* amphiploid, only the first occurrence is cited, in this case accompanied by a reference to a later review paper. Excluded from the list is the polyploid hybrid *T. polonicum* × *T. durum* (Zhebrak, 1940a), which is a typical autoploid rather than amphiploid. Also excluded is Bhatia's (1937) octoploid ($n = 28$) derivative from the hybrid *T. vulgare* × *T. dicoccum*. Doubled hybrids of Triticum species with different chromosome number, or with *T. timopheevi* involved, are included because the non-doubled F_1 is sterile or nearly so. Some of the amphiploids in Table 3 are described and discussed by Aase (1935, 1946).

No attempt is made to summarize the data on fertility, chromosome pairing, etc., in the amphiploids. Pairing is usually rather poor and fertility comparatively low where the amphiploid chromosome number is $n = 28$ or more. Kostoff (1939b, 1940) suggests that in genera which have large chromosomes, as do Triticum and Aegilops, mechanical difficulties in pairing may occur when the number of chromosomes is increased beyond a certain point. Peto and Boyes (1940) subscribe to this theory, also.

Lebedeff (1932), Oehler (1934), and Kostoff (1936c) have reported what Kostoff calls reduced allopolyploids — that is, fertile types with one or more genomes fewer than the combined genome complement of the two parent species. Lebedeff, in the F_2 of (*T. vulgare* × *S. cereale*) × *S. cereale*, obtained a plant thought to have seven pairs of rye chromosomes and seven pairs (presumably one full genome) of wheat chromosomes. Oehler's reduced allopolyploid was in the F_3 and F_4 of *Ae. triaristata* ($n = 21$) × *T. vulgare* ($n = 21$) and had 28 pairs of chromosomes of undetermined origin. Kostoff found a plant evidently of the genomic constitution *AABB* in the F_3 of *T. vulgare* × *T. monococcum* (diploid pollen).

Somewhat similar to reduced allopolyploids are types with the entire chromosome complement of one species plus one or more pairs of chromosomes (but less than an entire genome) from a second species. By backcrossing an amphiploid of hexaploid wheat × rye to its wheat parent, O'Mara (1940) succeeded in adding three different pairs of rye chromosomes separately to wheat. One of these three pairs was the so-called

"hairy-neck" chromosome, which various other investigators have added to wheat by backcrossing a wheat-rye F_1 to wheat. Kattermann (1938) obtained a substitution of this pair of hairy-neck chromosomes for a pair of wheat chromosomes. O'Mara (1946) substituted the hairy-neck pair for the speltoid (IXth) pair of wheat.

IV. Autoploids

Induced autoploids in the Triticinae have been notable chiefly for their low fertility and comparative lack of vegetative vigor. An exception is tetraploid *Secale cereale*, which flowers normally, except for an altered photoperiodic response (O'Mara, 1942) and sets a fair complement of seeds.

The first autoploids were induced by Dorsey (1936) and included *S. cereale*, *T. durum*, *T. polonicum*, and *T. vulgare*. Later (1939) Dorsey obtained a tetraploid of *T. monococcum*. This tetraploid produced no mature spikes. Kondo (1941) has added to the list of autoploids *Haynaldia villosa*, *Ae. bicornis*, *Ae. comosa*, *Ae. squarrosa*, and *Ae. uniaristata*. Matsumura (1940) induced an autoploid of *Ae. ovata*. Tumanien (1937) reported finding a tetraploid spike of *T. monococcum* in nature.

Several natural polyploids, presumably autoploids, occur in Agropyron (see Aase, 1946, for review), namely, *A. cristatum* (2×, 4×, 6× forms), *A. spicatum* (2×, 4×), *A. junceum* (4×, 6×), and *A. intermedium* (4×, 6×).

V. Haploids

Haploids have been reported in all three groups of the genus Triticum and in rye. They occur with frequencies approaching one per cent in diploid wheat (Katayama, 1934; Kihara, 1940c; Smith, 1936 and 1946b), but rarely in the other Triticum groups (Yamasaki, 1935) or in other genera of the Triticinae. Namikawa and Kawakami (1934) noted the occurrence of a haploid as one member of a twin seedling; and haploid members of twins have subsequently been noted by various investigators, including Müntzing (1938), who analyzed a large number of twins. The frequency of occurrence of haploids has been increased by X-ray treatment of pollen in *T. monococcum* by Katayama (1934) and in tetraploid wheats by Yefeikin and Vasilyev (1936). Kihara (1940c) and Smith (1946b), by delaying pollination until six or more days after emasculation, raised the frequency of haploids in *T. monococcum* from about one per cent to over 20 per cent.

Particular interest attaches to haploids because study of their meiotic behavior can indicate to what extent, if any, homology exists between the various chromosomes. In *T. monococcum* haploids an occasional bivalent

was observed by Katayama (1935), and Smith (1946b). In haploids of tetraploid wheats Kihara (1936) reported up to three pairs. In the hexaploids several investigators (Gaines and Aase, 1926; Kostoff, 1939a; Krishnaswamy, 1939; Raw, 1937; Yamasaki, 1936) have studied haploid meiosis. The maximum amount of association noted ranges from rarely more than one pair (Gaines and Aase, 1926) to as many as nine pairs (Krishnaswamy, 1939). It should be noted, however, that even in the material of Krishnaswamy (1939) the majority of the cells observed had no chromosome associations and only 0.4% had more than four pairs. It is probable that pairs in excess of four or five are the result of unusual circumstances — possibly the persistence of non-homologous prophase pairing, or possibly the occurrence of mutant cells or areas of cells with an increased number of chromosomes, as noted by Love (1938). The great majority of pairs observed are rod bivalents with one chiasma.

Haploid *Ae. ovata* showed pairing similar to that in *T. durum*, 3 bivalents being the maximum per cell (Matsumura, 1940).

In haploid rye Nordenskiöld (1939) found about 3% of pollen mother cells with one bivalent. Levan (1942) found considerably more pairing: over 20% of cells with one pair, and over 3% with two pairs. One cell (.03%) had all seven chromosomes associated (two bivalents and a trivalent); and several other combinations of bivalents and trivalents were noted. Since rye is a diverse, heterozygous plant, it is possible that Levan's material had one or more duplications not present in that of Nordenskiöld. Or perhaps circumstances were such as to favor either an unusually high frequency of association between small homologous segments or an unusual persistence of prophasic non-homologous associations. Levan concludes that at least the one frequently occurring pair must have been due to specific homology.

Offspring have been obtained from haploids of diploid and of hexaploid wheats. Katayama (1935) found three haploids and three normal diploids in *T. monococcum*, while Smith (1946b) found only two haploids among 90 offspring of haploids of the same species. In the hexaploid wheats fertility of haploids is higher, as many as 70 viable seeds having been obtained from a single plant following hand-pollination by normal plants (Sears, unpublished). Among the offspring, types with losses or additions of whole chromosomes and with reciprocal translocations predominate (Sears, 1939a, and unpublished). These irregularities presumably result from partial abortion of the first meiotic division in the haploid, and inclusion of most or all of the chromosomes in a restitution nucleus. The occurrence of restitution nuclei also accounts for the relatively high fertility of the haploid.

VI. Studies of Chromosome Morphology

In addition to the work on meiosis in various interspecific and intergeneric hybrids, various studies have been made of chromosome morphology in non-hybrid material. These studies, mostly made at mitotic metaphase, have chiefly been concerned with the demonstration of possible relationships between species and genera. In general, the method has been less successful than that of analyzing hybrids, although attention must be called to Pathak's (1940) successful prediction from Senjaninova-Korczagina's (1932) data that *Ae. squarrosa* might be proved the source of the *D* genome of hexaploid wheats.

A full treatment of the work on chromosome morphology is included in Aase's (1946) recent review.

VII. Cytogenetic Studies

Correlated cytological and genetical work in the Triticinae has been confined to the genus Triticum.

In the diploid wheats Smith (1936, 1939) described a number of reciprocal translocations and several X-ray induced mutants involving meiotic disturbances, and (1938) mentioned the occurrence of trisomics. Disjunction of adjacent chromosomes in translocation complexes was found to be the rule by Thompson and Thompson (1937) and Thompson and Hutcheson (1942).

In an F_2 generation of *T. durum* × *T. monococcum* Thompson (1931) recovered the monococcum condition for every character except density of spike. Since F_2 chromosome numbers (26 to 42) were mostly as great as, or greater than, that of the emmer parent, it was concluded that the *B* genome has little effect in determining the characteristics of tetraploid wheats.

The so-called pentaploid hybrids between hexaploid and tetraploid wheats, and their descendants, have been subjected to detailed cytogenetic study, beginning with the work of Sax (1923) and Kihara (1924). The researches of Kihara and Matsumura and others have indicated the factors involved in the obtaining in F_2 and first backcross of the observed frequencies of types with various numbers and combinations of *D*-genome chromosomes. Kihara and Matsumura (1940) summarize their findings approximately as follows:

1. Univalents tend to go to the poles in groups at second anaphase, thus increasing the proportion of 14- and 21-chromosome gametes (see also Watkins, 1940).
2. Univalents tend to be lost.
3. Eggs and especially pollen with intermediate chromosome numbers

tend to be eliminated. Pollen with 21 chromosomes is favored over that with 14.

4. Most zygotes formed by eggs with 14 chromosomes and pollen with around 21 chromosomes fail to survive.

5. Zygotes with from one to six *D* chromosomes or pairs are largely eliminated, except in certain crosses, notably *T. polonicum* × *T. spelta* (see also Thompson and Cameron, 1928).

6. Germination and viability are lower for embryos with low chromosome numbers.

Pao and Li (1943) have calculated various formulae expressive of the above findings.

Fertility in the F_2 generation, Kihara and Matsumura (1940) point out, is least for the types with intermediate chromosome numbers, becoming greater as the parental numbers are approached. Plants with 42 chromosomes are generally more fertile than those with 28.

In generations subsequent to F_2, according to the same authors, types with 29 to 34 chromosomes tend rapidly to revert to the number 28, while types with 36 to 41 chromosomes tend slowly to elevate their number to 42. Lines with only 40 chromosomes (20 pairs) are sometimes obtained (see also Love, 1940), but these apparently tend in time to acquire an additional pair through duplication of one of the 20 pairs present.

Cytogenetic analysis of F_2's, first backcrosses, and subsequent generations has indicated to a large extent which characteristics of hexaploid varieties actually depend on genes located in the *D* genome.

From a study of derived types with one or two chromosomes from the *D* genome of *T. spelta*, Yamashita (1937a) concluded that four of the *D* chromosomes have the following separate effects:

1. Causes red color in the young plant.
2. Causes narrow, somewhat curled leaves.
3. Decreases awn length and affects attitude of tillers.
4. Produces keel on glumes and causes hollow stems.

These observations are in accord with those of Watkins (1928), Thompson, Arnason, and Love (1935), and Arnason (1938) on the characteristics of hexaploid wheats which could not be transferred to tetraploids. Various other factors have been assigned to the *D* genome by these investigators — factors affecting density of spike, width and shape of rachis, glume shape and articulation of rachis, and location of hairs on rachis, keel, and leaf.

These characteristics dependent upon chromosomes of the *D* genome form a relatively small proportion of those studied in hexaploid-tetraploid hybrids. Some of these are dependent on factors in the *A* and *B* genomes as well as in the *D* genome. In addition there are many characteristics distinguishing most hexaploid varieties from most tetraploids which are

determined by factors in the *A* and *B* genomes rather than the *D*. Thompson (1925) was apparently the first to contend that factors determining or influencing vulgare characters are present in the *A* and *B* genomes as well as in the *D*, although McFadden (1930) had by 1924 developed vulgare varieties with the resistance of emmer to disease.

Nullisomics (that is, plants with only 20 pairs of chromosomes) in hexaploid wheat have been reported by several investigators, the best known type being homozygous speltoids of the so-called series *b*. Huskins (1946) has recently reviewed the literature on speltoids. Kihara (1939) has obtained nullisomics for four chromosomes of the *D* genome, following hybridization of *T. polonicum* × *T. spelta*. Other nullisomics have been reported by Thompson (1928), Uchikawa (1938), and Love (1940). Finally, Sears (1939a, 1944b) has obtained 17 different nullisomics, 11 for chromosomes of the *A* and *B* genomes and 6 for *D*-genome chromosomes. The 17 chromosomes concerned have been numbered from I to XI and from XV to XX, respectively. Except for the speltoid chromosome, which is numbered IX, no identification has been made of the nullisomics obtained by other investigators.

The nullisomics reported by Sears are all more or less depressed in vigor, but are all fertile to some extent either as male or female or both. They are obtained in frequencies of from less than 1% to more than 10% in the progenies of monosomics. By means of them several known factors have been identified with particular chromosomes, namely a factor for red seeds (chromosome XVI), the *sphaerococcum* factor (XVI), a factor for awn suppression (X), the "hooded" factor for shortened and recurved awns (VIII), and a factor for reduced synapsis (III). In addition, two previously unknown factors for promotion of awn growth are indicated (II and XX).

Tetrasomics have also been reported in hexaploid wheat (Huskins, 1931; Kihara, 1939; Sears, 1944b; Uchikawa, 1937). These are chiefly of interest in connection with studies on the effect of dosage of various genes. Tetrasomes can also serve, in combination with nullisomes, to indicate genetic homologies between various chromosomes. Kihara (1939) reported the occurrence of specific tetrasomes compensating partially or completely for each of the four nullisomes he was studying; and Huskins (1941) and Sears (1944b) have noted similar occurrences.

Isochromosomes (with identical arms) and telocentric chromosomes (with only one arm) appear in hexaploid wheat, especially following misdivision of monosomes (Huskins, 1946; Love, 1943; Sears, 1944b). These types permit the determination of which arm of a particular chromosome carries a particular genetic factor or complex of factors.

The frequency of chromosome aberrations following X-ray treatment

has been determined for the wheats by Fröier, Gelin, and Gustafsson (1941) and Smith (1943, 1946a). At dosages of 5000 to 10,000r on dormant seeds, the aberration frequency, as measured by the number of chromatin bridges per chromosome, is approximately the same in diploids, tetraploids, and hexaploids. Mutations following X-ray treatment were found by Stadler (1929) to vary in number inversely with the degree of polyploidy, presumably because in the polyploids there are duplications which obscure the effects of chromatin losses and other aberrations.

VIII. Genetic Studies

A. *Genetic Studies with Wheat*

Inheritance studies with wheat have dealt largely with the various hexaploid species and with hybrids between hexaploids and tetraploids. These hybrids (called pentaploids) are reasonably fertile and show fairly regular segregation for certain factors. Hybrids of diploid wheats with members of both other groups are sterile. The genetics of the diploids will be taken up separately, except for a few characters which closely resemble characters found in tetraploids or hexaploids.

No effort will be made to cite every paper dealing with each character; instead, reference will be made, wherever possible, to recent papers reviewing the earlier work. More complete literature citations for most of the characters discussed can be found in a recent review by Ausemus *et al.* (1946). The genetics of disease resistance will not be dealt with, since Ausemus (1943) has recently reviewed the large body of literature on this subject.

1. Diploid Wheats

The diploid wheats, or einkorns, have been subjected to intensive cytogenetic analysis by Smith (1936, 1939, 1942), who has described 56 viable mutants, mostly obtained through X-ray treatment. These are mostly chlorophyll deficiencies and morphological abnormalities, although they include several types with aberrant chromosome behavior, types with reduced pigmentation, and types with altered physiological patterns, such as increased earliness of maturity and increased susceptibility to X-ray injury. Sears (1944a) reported a naturally occurring factor determining viability of hybrids with *Aegilops umbellulata*. All but one of the 57 mutant types thus studied are dependent on single factors, and the other one involves two complementary genes. Smith (1939 and unpublished) has found linkages involving 25 of these factors. Six groups of from two to seven factors have been established, with map distances of up to 35 crossover units.

2. Tetraploid and Hexaploid Wheats

a. *Species-differentiating Characteristics.* Because fertile hybrids can easily be obtained between the various hexaploid and tetraploid species (excepting *T. timopheevi* and *T. armeniacum*), it has been possible to determine the genetic basis of several characters which are used in describing particular species or groups of species.

Solid culm. The hexaploid wheats are characterized by hollow, thin-walled culms. Some tetraploids have culms which are entirely or nearly filled with pith, while others have hollow but thick-walled culms. There appear to be two genes for hollow stems in the *A* and *B* genomes (Platt *et al*, 1941; Putnam, 1942; Yamashita, 1937b) and one in the *D* genome (Platt *et al*, 1941; Thompson *et al*, 1935; Watkins, 1930; Yamashita, 1937b). The three genes for hollowness are largely dominant and apparently are cumulative in action (Platt *et al*, 1941).

Elongate glumes. T. polonicum is characterized by glumes much longer than those of any other species. One partially dominant factor is largely or entirely responsible for these long glumes. Engledow (1923) failed to recover the full parental types in the F_2 of *T. polonicum* × *T. durum*, and Darlington (1927) attributed this "shift" to autosyndesis of *polonicum* and *durum* chromosomes carrying loci affecting glume length, with consequent maintenance of the heterozygous condition. The results of Liepin (1929), Malinowski (1925), and Almeida (1937), however, show that the apparent shift is more probably due to the action of two or more modifying factors.

Branched spike. Some forms of *T. turgidum* typically have ramified spikes, a characteristic which is dependent upon a single, recessive gene (von Tschermak, 1923). Under certain conditions of day-length and temperature, branched-spike behaves as a dominant character (Sharman, 1944a).

Glume shape and fragility of rachis. The early work of Watkins (1928) indicated that the differences among the tetraploid species in glume and rachis characteristics depended primarily upon a single pair of alleles, *K* and K^s. *K* gives loose glumes and a tough rachis to *T. durum* and *T. turgidum,* while K^s gives thick, tight glumes and a brittle rachis to *T. dicoccum* (and presumably to *T. dicoccoides*). Both lead to a strong keel. A third allele, *k*, presumably produces the non-keeled, loose glumes and tough rachis of *T. persicum.* These same three factors are responsible for the various glume characters of the hexaploid wheats, but their actions are modified somewhat by the presence of a factor, K^d (originally K', but changed by Watkins, 1940), in the *D* genome. This factor is similar to K^s in causing a thickening of the glumes, but differs in its effect on fragility of the rachis. It causes the rachis to break (when K^s is also present) just below the point of attachment of the spikelet to the rachis rather than

just above this point. Thus, *T. spelta* is $K^sK^s \cdot K^dK^d$, with thicker glumes and a rachis fracturing at a different place than in *T. dicoccum*. $KK \cdot K^dK^d$ is the speltoid type, which has thick, tight glumes rather than the loose glumes of its tetraploid counterparts, *T. durum* and *T. turgidum*. Both these types are strongly keeled, like the tetraploids. *T. vulgare* is $kk \cdot K^dK^d$ and has non-keeled, loose glumes, and a tough rachis, as in the tetraploid *T. persicum*.

From the preceding paragraph it is clear that the three K alleles in the A or B genome are responsible for the major differences between three tetraploid and three hexaploid types, all but one of these types being of specific rank. It should be noted that of the three factors concerned, K is evidently a deficiency for the locus, since speltoidy occurs following loss of the chromosome carrying this locus. It should also be noted that the K^d "factor" has not actually been identified as a single factor, but may represent the combined effect of a number of factors within the D genome (*i.e.*, the genome of *Ae. squarrosa*). A single factor is assumed because of the common occurrence of duplicate or near-duplicate factors in polyploid species.

In a subsequent paper, Watkins (1940) has proposed another pair of alleles, K and K^d, located in the A or B genome independently of the K, K^s, k series. This third locus is introduced to account for the anomalous behavior of a *T. turgidum* × *T. dicoccum* cross in which the F_2 failed to segregate for glume characters, all plants resembling the F_1. It is suggested that *T. dicoccum* is $K^dK^d \cdot K^dK^d$ and *T. turgidum* $KK \cdot KK$, and that autosyndesis involving the chromosomes carrying these factors occurs in the hybrid. All the other wheats discussed are held to carry KK at the third locus. *T. vulgare* is then $kk \cdot KK \cdot K^dK^d$, and the chromosome carrying K^d remains unpaired in hybrids with *T. turgidum* and that carrying K unpaired in hybrids with *T. dicoccum*. *T. spelta* is $K^sK^s \cdot KK \cdot K^dK^d$.

This interesting hypothesis of a third K locus explains the anomalous *dicoccum-turgidum* hybrid satisfactorily, but gives rise to other difficulties, among which may be mentioned the following: First, *T. spelta* is equivalent to *T. dicoccum* × *Ae. squarrosa* (McFadden and Sears, 1946). This would make *T. spelta* $K^dK^d \cdot K^dK^d \cdot K^dK^d$ instead of $K^sK^s \cdot KK \cdot K^dK^d$. Second, if autosyndesis occurs regularly in *turgidum-dicoccum* hybrids, as the theory states, then quadrivalents should occur frequently in the parent species; whereas they have been observed but rarely (Hosono, 1935). Third, wide hybrids involving either *T. turgidum* or *T. dicoccum* should regularly show at least one pair of chromosomes at meiosis, whereas several such hybrids have a mode of 0 pairs.

On the basis of the evidence now available, Watkins' original theory seems more acceptable than his modified hypothesis. Sharman's (1944b)

study of F_1 hybrids bears out Watkins' theories on the keel, though his data do not furnish critical evidence on the relative validity of Watkins' two theories.

The sphaerococcum character. Ellerton (1939) has shown that the complex of characters associated with *T. sphaerococcum* (short culms, very compact spike, and nearly spherical seeds) is based on a single, largely recessive, genetic factor. Sears (1946) finds this factor to be located on chromosome XVI, and notes that, though recessive, it is a positively acting gene with effect dependent on dosage.

The compactum character. T. compactum also has short culms and very compact spikes, but has relatively normal seeds. Early work (see Kajanus, 1927) showed the condition to depend on a single, largely dominant factor, *C*, inherited independently of the *K* factors.

b. *Awn Characteristics. Awn length.* The work of Watkins and Ellerton (1940) shows three or possibly four main loci affecting length of awn. At one locus, B_1 and b_1^a reduce the awns to tip-awns or half-awns, respectively, and are largely dominant over the non-inhibitory allele, b_1. At another locus are B_2 and b_2, similar in effect to B_1 and b_1. A factor b_2^a or *A*, similar to b_1^a, probably belongs to this series, also, but may lie at a separate locus. Finally, *Hd* (hooded) reduces the awns and makes them curved or twisted. All of these factors are in the *A* and *B* genomes. Sears (1944b) has located two factors (or groups of factors) which increase awn length, one in the *A* or *B* genome and the other in the *D* genome. No alleles are known at these loci, their existence having been detected through chromosomal deficiencies. Although the *D* genome contains an awn-promoting factor, its general effect is to reduce awn length (Watkins and Ellerton, 1940).

In addition to the major awn genes described above, there are several other factors (see Watkins and Ellerton, 1940, for summary) which affect awn length to a greater or less extent, although their main effect is on some other character, such as spike density or glume length.

Awn on outer glume. T. persicum differs from other wheats in having an awn or tooth on the outer glume. Liepin (1930) obtained evidence for the existence in *T. durum* of multiple factors suppressing this awn. In diploid wheat Smith (1939) reported an X-ray induced, single-factor, recessive mutant with an awn on the outer glume.

Awn barbing. Knowles (1943) and Knowles and Harrington (1943) attribute the presence of full-length barbs on awns to one major gene. A minor barbing gene is capable of producing short barbs in the absence of the major gene. Both are located in the *A* or *B* genome, and are affected to some extent by modifiers.

c. *Pubescence. Pubescent node.* There is evidently a single, dominant

gene for pubescent node (Gaines and Carstens, 1926), located in the *A* or *B* genome.

Pubescent glume. Two independent, dominant factors for pubescence are known, one producing long hairs and the other relatively short hairs (Howard and Howard, 1912, 1915). Vacenko (1934) finds at one locus two alleles for different hair lengths, in addition to the allele for absence of hairs. Smith (1939) in diploid wheat describes a single, dominant factor for glume pubescence.

Pubescent leaf. Two independent, duplicate dominants for leaf pubescence have been reported (Neatby and Goulden, 1930).

d. *Color. Coleoptile and culm color.* There are two, dominant factors, either able to produce red coleoptile. At least one of these genes can cause a red (or purple) culm, for Worzella (1942) found complete association between colored coleoptile and purple culms. Varieties exist, however, which have colored coleoptiles but not colored culms. Quisenberry (1931) reported two factors for colored coleoptile in the variety H-44. In diploid wheats Smith (1939) found a single dominant factor for red coleoptile.

Auricle color. Kajanus (1927) reports a single, dominant gene for red over colorless.

Glume color. Kajanus (1927) classifies the glume-colors of the wheat species and varieties as black, red-brown, yellow-brown and yellow. Yellow is the multiple recessive type. The available genetic data can be interpreted on the basis of three independent factors. Black differs from yellow by a single, dominant gene. Red-brown and yellow-brown each differ from yellow by a single, dominant factor, both factors being hypostatic to black. Evidently all three of these factors are located in the *A* and *B* genomes. Classification is often difficult. In diploid wheats a single, dominant gene for black glumes was found by Smith (1939).

Awn color. A single factor for black awns over yellow was noted by Sax and Gaines (1924). It seems likely that this factor is the same as the one causing black glumes. The black-awned type (*T. turgidum*) used by Sax and Gaines also had black glumes, but no classification in F_2 and F_3 could be made for glume color. Color in the glumes is more readily inhibited by environmental and presumably genetic factors than is awn color. Several varieties of *T. durum* have black awns and yellow glumes, but no wheat varieties have black glumes and yellow awns.

Anther color. Sharman (1944c) reports a single dominant factor for purplish-pink color in anthers.

Seed color. The classical experiments of Nilsson-Ehle (1911) showed that three additive, partially dominant factors were responsible for the various degrees of red-brown coloration exhibited by the pericarp of the

seed. In certain varieties of *T. dicoccum* and *T. durum* there are evidently additional seed-color factors, particularly for purple color (Clark, 1936).

e. *Ligule.* Presence of a ligule is conditioned by two duplicate, dominant factors (Kulkarni, 1934).

f. *Waxy Bloom.* *T. vulgare* and *T. turgidum* both have waxy bloom, but segregation occurs in the F_2 of their hybrid. This is attributed (Watkins, 1927) to the fact that *turgidum* has a dominant factor for waxiness, while *vulgare* has a recessive, non-waxy allele at this locus and a dominant gene for waxiness in the *D* genome.

g. *Growth Habit.* Spring type is dominant over winter. Three factors, somewhat cumulative in action, were found by Åkerman (1943) in a survey of varieties. Earliness evidently depends upon several factors (Gfeller, 1937).

h. *Size.* *Length of culm.* There are undoubtedly a number of factors affecting length of culm. This is clearly indicated by the dwarfness of the majority of the nullisomic types (Sears, 1944b). Most of the factors affecting density of spike also affect culm length. Pao, Li, Chen, and Li (1944) postulate seven loci as being involved in the production of a certain type of dwarf. Other investigators (Florell and Martin, 1936; Goulden, 1926; McMillan, 1937; Thompson, 1928) have also found types of dwarfness dependent upon two or more factors.

Breadth of culm and leaf. Von Tschermak (1923) found thickness of culm to be due to several factors, and Freeman (1919) reached a similar conclusion concerning leaf width. These findings agree with the data from the nullisomics, where thin-stemmed, narrow-leaved types predominate.

Density of spike. The *sphaerococcum* and *compactum* factors previously mentioned increase the density of the spike, and the *spelta* factor (K^s) decreases density slightly. In addition, there is a recessive, "squarehead" factor *q* which results in a shortening of the rachis internodes in the upper portion of the spike (Raum, 1931). This factor is located on the speltoid (IXth) chromosome (Philiptschenko, 1934). Philiptschenko (1930) proposed a second factor for squareheadedness, but the evidence seems inconclusive that this factor is not the same as *q*. In addition to these factors there are evidently at least two dominant factors for lengthening of internodes and a number of modifying factors with relatively small effects on spike density (Boshnakian, 1922; Mudra, 1939; Nilsson-Ehle, 1911; Philiptschenko, 1929; Raum, 1931).

Breadth of glume. Philiptschenko (1928) postulated one main factor and 10 modifiers to explain inheritance of glume width in crosses among hexaploid wheats. Tai (1934) reported two complementary dominants for narrow glume-shoulders in a hexaploid cross.

Kernel weight. Several factors are evidently concerned in determining

weight of kernel (Worzella, 1942). Sax (1921) noted increased size of cross-pollinated seeds in the hexaploid group and in the tetraploid group. Griffee (1921) also observed increased seed size following crosses among hexaploid varieties.

i. *Composition of Seed.* Hardness, gluten strength, crude protein content, and content of carotenoid pigment each depends upon multiple factors (Worzella, 1942).

j. *Shattering.* Howard and Howard (1912) found a two-factor difference for shattering in *compactum* × *vulgare.*

k. *Lodging.* A number of factors are concerned with lodging (Nilsson-Ehle, 1913).

l. *Supernumerary Spikelets.* A tendency toward the production of extra spikelets inserted immediately below the normal spikelets is inherited as a simple recessive (Kajanus, 1927). The degree of expression depends to a large extent on environment.

m. *Chlorophyll Deficiency.* From studies of induced mutation (Fröier, 1946; Smith, 1936, 1939), it is clear that in diploid wheats many different genes are involved in the development of normal chlorophyll. In the tetraploid wheats most of these genes are present in duplicate, as evidenced by the relative difficulty of inducing types with aberrant chlorophyll development (Fröier, 1946; Stadler, 1929), and by the fact that most of the known mutants depend on two complementary recessives (Harrington and Smith, 1928; Kihara, 1937b, and others — see Fröier, 1946, for review). Goytia (1935), however, found in *T. durum* a late-appearing, yellow condition determined by a single, dominant gene; and Fröier (1946) obtained two single-factor recessives through X-ray treatment.

The hexaploids have a still higher degree of duplication, as shown by Stadler's (1929) and Fröier's (1946) inability to induce any chlorophyll mutations whatever, and by the absence of chlorophyll defects among nullisomics (Sears, 1944b). Neatby (1933) found a virescent-yellow type in *T. vulgare* which behaved as a single-factor recessive. This mutation involved more than merely the loss of a locus not compensated for elsewhere in the gene complement, for Neatby observed reverse mutations to normal, as well as mutations to albino.

Maternally inherited variegation in *T. vulgare* has been described by Umar (1943) and Pao and Li (in press).

n. *Physiological Characteristics. Crossability with rye.* A Chinese variety of *T. vulgare* crosses readily with *Secale cereale* (Backhouse, 1916). The results of Taylor and Quisenberry (1935) and Lein (1943) indicate that this crossability is due to one recessive major factor and at least one recessive minor factor.

Meiotic instability. There is at least one major, dominant factor in

hexaploid wheats for normal synapsis. Li, Pao, and Li (1945) have found a case of partial desynapsis due to a single recessive factor, and Sears (1944b) has described a similar condition associated with a particular chromosome deficiency. The existence of minor factors affecting meiosis is suggested by the work of Myers and Powers (1938).

Phenol reactivity. Miczynski (1938) finds one dominant factor which leads to the reaction of phenol with glumes to produce coloration. This factor and one other determine the corresponding reactivity of seeds. Voss (1938) believes that these differences in reactivity depend on differences in tyrosinase activity.

Lethality of hybrids. Certain varietal hybrids among hexaploid wheats die as seedlings. Caldwell and Compton (1943) and Heyne, Wiebe, and Painter (1943) found two dominant, complementary factors responsible. That a different pair of complementary factors may be involved in each of several different lethal combinations is indicated by the work of Kostyuchenko (1936). "Firing," a sublethal condition in which leaves and sheaths die at about flowering time, is reportedly dependent on three complementary dominant factors (McMillan, 1936).

Winterhardiness. Winterhardiness depends on several physiological and morphological characteristics (Salmon, 1917), and is complex in inheritance (Quisenberry and Clark, 1929). One important component, cold resistance, itself depends on several genes (see Worzella, 1942).

o. *Linkages.* Several factors are known to lie on the speltoid (IX) chromosome. The glume-shape factors K, K^s and k are located about 30 cross-over units from the awn suppressor B_1 (see Watkins and Ellerton, 1940), while the gene q for squarehead spike type lies about 35 units on the other side of B_1 (Philiptschenko, 1934). The factor for pubescent node is located about 5 units from B_1 (Gaines and Carstens, 1926).

Much seemingly conflicting evidence has been reported concerning the linkage of glume pubescence and glume color. After reviewing this evidence, however, Kajanus (1927) concludes that black color and one factor for pubescence are completely linked (supporting the existence of this linkage are Smith's (1939) similar results with varieties of diploid wheat), while red-brown and the other pubescence factor are strongly linked. In certain crosses the factor for black color (and associated pubescence) appears to be linked with the recessive alleles of red-brown and the second pubescence factor, but in other crosses no association with them is indicated.

Worzella (1942) observed an association between glume color and gluten strength in crosses between varieties of *T. vulgare*. This association was attributed to a linkage, with crossover value of about 35 per cent, between the factor for glume color and a factor for gluten strength. Dis-

turbingly enough, no closer association was found in the cross where gluten strength was apparently monofactorially determined than where it gave evidence of depending on four independent factors. There is, however, no obvious explanation for the association other than linkage.

An association between kernel color and crude protein content was also noted by Worzella (1942) and was attributed to a linkage, with cross-over value of about 28 per cent, between a kernel-color factor and a gene for high protein. Although the work of Nilsson-Ehle (1914) showed that in red seeds the inner layer of the pericarp is considerably thicker than in white seeds, there appears to be little possibility that the increased amount of pericarp material in red seeds results directly in an increase in total protein. The analyses of Teller and Teller (1932) indicate that the pericarp of wheat is little, if any higher in protein content than is the remainder of the kernel. The bran (pericarp plus part of the aleurone layer) of red wheats was no higher than, if as high as, that of white wheats in protein. It is possible that genes for red color themselves increase protein content while at the same time increasing the amount of pigment in the pericarp. If this is true, the correlation should be as high when all of the three known color genes are segregating as when only one is segregating as in Worzella's cross. Another of Worzella's crosses did involve a three-factor difference for seed color, but no data are given for the association of color with protein content — perhaps because so few white-seeded segregates (only four) were obtained. The fact that some white wheats are high in protein content (Shollenberger and Clark, 1924) tends to support Worzella's linkage hypothesis.

B. *Genetic Studies with Aegilops and Secale*

Little genetic work has been done in the Triticinae other than with Triticum. In the genus Aegilops many interspecific crosses have been made, but since all the hybrids are nearly or completely sterile, genetic analysis of species-determining characters has not been possible. The differences between the varieties *ligustica* and *aucheri* of *Ae. speltoides* are apparently due to a single factor (Kihara and Lilienfeld, 1932). Kihara (1937b) obtained monofactorial albino and xantha types in *Ae. caudata* following crossing with *Ae. cylindrica* and backcrossing to *caudata*. In *Ae. triuncialis* blackness and pubescence of glumes are each dependent on a single, dominant factor (Miczynski, 1941). Waxy bloom in *Ae. ventricosa* evidently involves one factor for presence of bloom and one dominant inhibitor of bloom (Iwata, 1938; Miczynski, 1941). One of these factors is reportedly linked with the pubescence factor (Miczynski, 1941).

Genetic studies with rye have revealed a few mendelian factors. Absence of waxy bloom is a simple, recessive characteristic (Heribert-

Nilsson, 1921). A condition known as "brittle stem" depends on a single, recessive gene (Brewbaker, 1926; Jermoljev, 1942), as does liguleless (Krasniuk, 1936a). One dominant factor results in a brown grain and faintly red coleoptile, while a second dominant factor interacts with the first to produce a green seed and red coleoptile (Dumon, 1938). Several different types of chlorophyll deficiency, each dependent upon a single, recessive gene, were described by Brewbaker (1926). Wagner (1942) found three different types of maternally inherited variegation, which could be transmitted through the pollen by means of a sufficient number (four or five) of backcrosses. Fertility of open-pollinated rye plants varies considerably, and a unit factor affecting this fertility has been described (Krasniuk, 1936b). Rye is normally self-sterile, but various investigators (Brewbaker, 1926; Leith and Shands, 1938; Ossent, 1938) have succeeded in raising self-fertility to useful levels by selection. Multiple factors are apparently involved here. A type of asynapsis in rye has been found due to a single, recessive gene (Prakken, 1943).

IX. REFERENCES

Aase, H. C. *Bot. Rev.* **1,** 467–496 (1935).

Bot. Rev. **12,** 255–334 (1946).

Åkerman, Å., *Sverig. Utsädesfören. Tidskr.* **53,** 51–66 (1943); *Plant Breed. Abstr.* **14,** 173.

Almeida, J. R. M. de., *Rev. agron., Lisboa* **25,** 38–55 (1937).

Armstrong, J. M., *Emp. J. exp. Agric.* **13,** 41–53 (1945).

Arnason, T. J., *Canad. J. Res. C* **16,** 174–181 (1938).

Ausemus, E. R., *Bot. Rev.* **9,** 207–260 (1943).

Ausemus, E. R., Harrington, J. B., Reitz, L. P., and Worzella, W. W., *J. Amer. Soc. Agron.* **38,** 1082–1099 (1946).

Backhouse, W. O., *J. Genet.* **6,** 91–94 (1916).

Bhatia, G. S., *J. Genet.* **35,** 331–349 (1937).

Boshnakian, S., *Mem. Cornell Univ. agric. Exp. Sta.* **53,** 801–882 (1922).

Brewbaker, H. E., *Tech. Bull. Minn. agric. Exp. Sta.* **40,** pp. 40 (1926).

Britten, E. J., and Thompson, W. P., *Science* **93,** 479 (1941).

Caldwell, R. M., and Compton, L. E., *J. Hered.* **34,** 67–70 (1943).

Camara, A., *Ann. Inst. Sup. Agron., Lisboa* **7,** 29–61 (1935).

Agrononomia Lusitana **5,** 95–117 (1943).

Clark, J. A., *Yearb. Agric. U. S. Dep. Agric.* 1936, 207–302 (1936).

Clausen, J., Keck, D. D., and Hiesey, W. M., *Publ. Carneg. Instn.* **564,** pp. 174 (1945).

Darlington, C. D., *Nature, Lond.* **119,** 390–391 (1927).

Derzhavin, A., *Social. Reconstr. Agric.* 1938, 213–219 (1938); *Plant Breed. Abstr.* **9,** 180.

Dorsey, E., *J. Hered.* **27,** 155–160 (1936).

J. Hered. **30,** 393–395 (1939).

Dumon, A. G., *Agricultura, Louvain* **41,** 190–196 (1938); *Plant Breed. Abstr.* **9,** 718.

Eig, A., *Repert. nov. Spec. Regn. veg.* **55,** 1–228 (1929).

Ellerton, S., *J. Genet.* **38,** 307–324 (1939).

Engledow, F. L., *J. Genet.* **13,** 79–100 (1923).

Favorsky (Favorskii), N. V., *Social. Grain Farming, Saratov* **5,** 115–125 (1935). (Russian with English summary.)
Social. Grain Farming, Saratov **6,** 95–101 (1936); *Plant Breed. Abstr.* **7,** 157
Florell, V. H., and Martin, J. F., *J. agric. Res.* **53,** 151–160 (1936).
Freeman, G. F., *Genetics* **4,** 1–93 (1919).
Fröier, K., *Hereditas, Lund,* **32,** 297–406 (1946).
Fröier, K., Gelin, O., and Gustafsson, Å., *Bot. Notiser.* 1941, 199–216 (1941).
Gaines, E. F., and Aase, H. C., *Amer. J. Bot.* **13,** 373–385 (1926).
Gaines, E. F., and Carstens, A., *J. agric. Res.* **33,** 753–755 (1926).
Gfeller, F., *Sci. Agric.* **17,** 482–491 (1937).
Goulden, C. H., *Tech. Bull. Minn. agric. Exp. Sta.* **33,** pp. 37 (1926).
Goytia y Angulo, M. de., *Bol. Inst. Invest. agron., Madrid* **1,** 153–163 (1935); *Plant Breed. Abstr.* **6,** 501.
Griffee, F., *J. agric. Res.* **22,** 53–63 (1921).
Harrington, J. B., and Smith, W. K., *Sci. Agric.* **9,** 147–153 (1928).
Heribert-Nilsson, N., *Hereditas, Lund,* **2,** 364–369 (1921).
Heyne, E. G., Wiebe, G. A., and Painter, R. H., *J. Hered.* **34,** 243–245 (1943).
Hosono, S., *Jap. J. Bot.* **7,** 301–322 (1935).
Howard, A., and Howard, G., *Mem. Dep. Agric. India Bot.* **5,** 1–47 (1912).
Mem. Dep. Agric. India Bot. **7,** 273–285 (1915).
Huskins, C. L., *J. Genet.* **25,** 113–124 (1931).
Amer. Nat. **75,** 329–344 (1941).
Bot. Rev. **12,** 457–514 (1946).
Iwata, K., *Jap. J. Genet.* **14,** 159–171 (1938); *Plant Breed. Abstr.* **9,** 216.
Jermoljev, E., *Z. Pflanzenz.* **24,** 1–58 (1942); *Plant Breed. Abstr.* **15,** 983.
Kajanus, B., *Bibliogr. genet.* **3,** 141–244 (1927).
Kasparyan, A. S., *C. R. Acad. Sci. U.R.S.S.* **26,** 166–169 (1940).
Katayama, Y., *Cytologia, Tokyo* **5,** 235–237 (1934).
Jap. J. Bot. **7,** 349–380 (1935).
Kattermann, G., *Z. indukt. Abstamm.-u. VererbLehre.* **74,** 354–375 (1938).
Khijnyak (Hizniak), V. A., *C. R. Acad. Sci. U.R.S.S.* **17,** 489–490 (1937).
Kihara, H., *Mem. Coll. Sci. Kyoto B,* 1–200 (1924).
Agric. Hort., Jap. **11,** 1425–1434 (1936), *Plant Breed. Abstr.* **7,** 609.
Mem. Coll. Agric. Kyoto **41,** pp. 61 (1937a).
Bot. Mag., Tokyo **51,** 584–589 (1937b).
Bot. Zool., Japan **7,** 211–218 (1939); *Plant Breed. Abstr.* 15, 183.
Züchter **12,** 49–62 (1940a).
Jap. J. Genet. **16,** 309–320 (1940b); *Plant Breed. Abstr.* 14, 1190.
Bot. Mag., Tokyo **54,** 178–185 (1940c).
Agric. Hort., Japan **19,** 889–890 (1944).
[The classification of *Aegilops* in the light of genome assay.] Chart distributed by Kihara (1945).
Kihara, H., and Lilienfeld, F., *Cytologia, Tokyo* **3,** 384–456 (1932).
Kihara, H., and Matsumura, S., *Jap. J. Bot.* **11,** 27–39 (1940).
Knowles, P. F., *Canad. J. Res. C* **21,** 198–204 (1943).
Knowles, P. F., and Harrington, J. B., *Sci. Agric.* **23,** 697–707 (1943).
Kondo, N., *Jap. J. Genet.* **17,** 46–54 (1941); *Plant Breed. Abstr.* **13,** 477.
Kostoff, D., *Z. indukt. Abstamm.- u. VererbLehre.* **72,** 115–118 (1936a).
Z. Pflanzenz. **21,** 41–45 (1936b).
Bull. Acad. Sci. U.R.S.S., Cl. Sci. Math. Nat., Ser. Biol. 1936c **(1), 5–22 (1936c).**

Kostoff, D. (*continued*)
C. R. Acad. Sci. U.R.S.S. **14,** 213-214 (1937).
C. R. Acad. Sci. U.R.S.S. **24,** 479-482 (1939a).
Biodynamica **51,** 1-14 (1939b).
J. Hered. **31,** 33-34 (1940).
Kostoff, D., and Arutiunova, N., *Curr. Sci.* **5,** 414-415 (1937).
Kostyuchenko, I. A., *Bull. appl. Bot. Leningrad, Ser. A* **(19),** 127-137 (1936); *Plant Breed. Abstr.* **8,** 99.
Kovaleva, P. G., *Hybridization* 1941 (2), 99-100 (1941); *Plant Breed. Abstr.* **14,** 1199.
Krasniuk, A. A., *Breed. Seed Growing* 1936, 50-53 (1936a); *Plant Breed. Abstr.* **7,** 972.
Social. Reconstruct. Agric. 1936, 104-112 (1936b); *Plant Breed. Abstr.* **8,** 828.
Krishnaswamy, N., *Hereditas, Lund* **25,** 77-86 (1939).
Kulkarni, L. G., *J. Amer. Soc. Agron.* **26,** 885-893 (1934).
Lebedeff, V. N., *Ukrain. sci. Inst. Sugar-Beet Industry, Kiev* (1932). [Cited by Kostoff in *Bull. Acad. Sci. U.R.S.S., Cl. Sci. Math. Nat., Ser. Biol.* 1936 (1), 5-22.]
Lein, A., *Z. indukt. Abstamm.- u. VererbLehre.* **81,** 28-59 (1943); *Plant Breed. Abstr.* **14,** 1197.
Leith, B. D., and Shands, H. L., *J. Amer. Soc. Agron.* **30,** 406-418 (1938).
Levan, A., *Hereditas, Lund* **28,** 177-211 (1942).
Li, H. W., Pao, W. K., and Li, C. H., *Amer. J. Bot.* **32,** 92-101 (1945).
Liepin, T., *Bull. Bur. Genet. (Izv. Byu. Genet.)* **7,** 41-68 (1929).
Bull. Bur. Genet. (Izv. Byu. Genet.) **8,** 19-45 (1930).
Lilienfeld, F., and Kihara, H., *Cytologia, Tokyo* **6,** 87-122 (1934).
Lindschau, M., and Oehler, E., *Züchter* **7,** 228-233 (1935).
Ljubimova, V. F., *Ogiz-Selkhozgiz* 1937, 211-223 (1937); *Plant Breed. Abstr.* **9,** 189.
Longley, A. E., and Sando, W. J., *J. agric. Res.* **40,** 683-719 (1930).
Love, R. M., *Genetics* **23,** 517-522 (1938).
Canad. J. Res. C **18,** 415-434 (1940).
Canad. J. Res. C **19,** 351-369 (1941).
Canad. J. Res. C **21,** 257-264 (1943).
Love, R. M., and Suneson, C. A., *Amer. J. Bot.* **32,** 451-456 (1945).
McFadden, E. S., *J. Amer. Soc. Agron.* **22,** 1020-1034 (1930).
McFadden, E. S., and Sears, E. R., *Rec. Genet. Soc. Amer.* **13,** 26-27 (1944); *Genetics* **30,** 14 (1945).
J. Hered. **37,** 81-89, 107-116 (1946).
J. Amer. Soc. Agron. **39,** 1011-1026 (1947).
McMillan, J. R. A., *J. Coun. sci. industr. Res., Australia* **9,** 283-294 (1936).
Bull. Coun. sci. industr. Res., Australia **104,** pp. 68 (1937); *Plant Breed. Abstr.* **8,** 13.
Malinowski, E., *Ann. Sci. Agr., Poznan* **14,** 1-8 (1925).
Matsumura, S., *Bot. Mag., Tokyo* **54,** 404-413 (1940).
Miczynski, K., *Z. Pflanzenz.* **22,** 564-587 (1938); *Plant Breed. Abstr.* **9,** 697.
Proc. 7th Int. Congr. Genet., **219** (1941).
Mudra, A., *Bull. Fac. agron. Cluj.* **7,** 176-192 (1939); *Plant Breed. Abstr.* **10,** 103.
Müntzing, A., *Hereditas, Lund* **24,** 487-491 (1938).
Hereditas, Lund **25,** 387-430 (1939).
Myers, W. M., and Powers, L., *J. agric. Res.* **56,** 441-452 (1938).
Namikawa, S., and Kawakami, J., *Proc. Imp. Acad., Tokyo* **10,** 668-671 (1934).
Neatby, K. W., *J. Hered.* **24,** 159-162 (1933).
Neatby, K. W., and Goulden, C. H., *Sci. Agric.* **10,** 389-404 (1930).

Nevsky, S. A., *Trudy Bot. Inst. Akad. Nauk. SSSR.*, Ser. I (**2**), 33–90 (1936). [Russian with German summary, p. 90.]; *Herb. Abstr.* **7**, 99.
Nilsson-Ehle, H., *Acta Univ. lund.*, N. F., Afd. **2**, Bd. 7, 3–84 (1911).
Beitr. Pflanzenz. **3**, 62–88 (1913).
Z. Pflanzenz. **2**, 153–187 (1914).
Nordenskiöld, H., *Hereditas, Lund* **25**, 204–210 (1939).
Oehler, E., *Züchter* **6**, 263–270 (1934).
Züchter **8**, 29–33 (1936).
O'Mara, J. G., *Genetics* **25**, 401–408 (1940).
Amer. Nat. **76**, 386–393 (1942).
Rec. Genet. Soc. Amer. **15**, 62–63 (1946); *Genetics* **32**, 99–100 (1947).
Östergren, G., *Hereditas, Lund* **26**, 395–398 (1940).
Ossent, H. P., *Züchter* **10**, 255–261 (1938).
Pao, W. K., Li, C. H., Chen, C. W., and Li, H. W., *J. Amer. Soc. Agron.* **36**, 417–428 (1944).
Pao, W. K., and Li, H. W., *Chinese J. Agric.* **1**, 23–35 (1943).
Pao, W. K., and Li, H. W., (In press).
Pathak, G. N., *J. Genet.* **39**, 437–467 (1940).
Peto, F. H., *Canad. J. Res. C* **14**, 203–214 (1936).
Canad. J. Res. C **16**, 516–529 (1938).
Genetics **24**, 93 (1939).
Peto, F. H., and Boyes, J. W., *Canad. J. Res. C* **18**, 230–239 (1940).
Philiptschenko, J., *Z. indukt. Abstamm.- u VerebLehre.*, Suppl. 2, 1184–1192 (1928).
Biol. Zbl. **49**, 1–16 (1929).
Bull. Bur. Genet. (*Izv. Byu. Genet.*) **8**, 1–18 (1930).
Genetics of soft wheat. Moscow. (1934). [Cited by Watkins and Ellerton, *J. Genet.* **40**, 243–270 (1940).]
Platt, A. W., Darroch, J. G., and Kemp, H. J., *Sci. Agric.* **22**, 216–224 (1941).
Prakken, R., *Hereditas* **29**, 475–495 (1943); *Plant Breed. Abstr.* **13**, 1238.
Putnam, L. G., *Sci. Agric.* **22**, 594–607 (1942).
Quisenberry, K. S., *Tech. Bull. U. S. Dep. Agric.* **218**, pp. 45 (1931).
Quisenberry, K. S., and Clark, J. A., *Tech. Bull. U. S. Dep. Agric.* **136**, pp. 28 (1929).
Raum, J., *Z. Pflanzenz.* **16**, 161–254 (1931).
Raw, A. R., *J. Dept. Agric., Victoria* **35**, 300–306 (1937).
Salmon, S. C., *J. Amer. Soc. Agron.* **9**, 353–380 (1917).
Sando, W. J., *J. Hered.* **26**, 229–232 (1935).
Sapehin, A. A., *J. Bot. U.R.S.S.* **20**, 119–125 (1935).
Sax, K., *Genetics* **6**, 399–416 (1921).
Genetics **8**, 301–321 (1923).
Sax, K., and Gaines, E. F., *J. agric. Res.* **28**, 1017–1032 (1924).
Schiemann, E., *Handb. Vererbungsw.* 3(L), p. 377 (1932).
Sears, E. R., *Genetics* **24**, 509–523 (1939a).
J. Hered. **30**, 38–43 (1939b).
Res. Bull. Mo. Agric. Exp. Sta. **336**, p. 46 (1941).
Genetics **29**, 113–127 (1944a).
Genetics **29**, 232–246 (1944b).
J. agric. Res. **68**, 135–144 (1944c).
Rec. Genet. Soc. Amer. **15**, 65–66 (1946); *Genetics* **32**, 102–103 (1947).
Senjaninova-Korczagina, M., *Bull. appl. Bot., Genet., Plant Breed.*, Ser. **2**(1), 1–90 (1932).
Sharman, B. C., *Nature, London* **153**, 497–499 (1944a).

Sharman, B. C. (*continued*)
Proc. Leeds phil. Soc. **4,** 215–221 (1944b).
Nature, London **154,** 675 (1944c).
Shollenberger, J. H., and Clark, J. A., *Bull. U. S. Dep. Agric.* **1183,** p. 94 (1924).
Sipkov, T. P., *Bull. appl. Bot., Leningrad,* Ser. **2**(9), 357–360 (1936).
Smith, D. C., *J. agric. Res.* **64,** 33–47 (1942).
Smith, L., *Res. Bull. Mo. Agric. Exp. Sta.* **248,** pp. 38 (1936).
Genetics **23,** 168 (1938).
Res. Bull. Mo. Agric. Exp. Sta. **298,** pp. 26 (1939).
Amer. J. Bot. **29,** 189–191 (1942).
J. Hered. **34,** 131–134 (1943).
J. agric. Res. **73,** 137–158 (1946a).
J. agric. Res. **73,** 291–301 (1946b).
Sorokina, O. N., *Bull. appl. Bot., Genet., Plant Breed.,* Ser. **2**(7), 161–173 (1937).
Stadler, L. J., *Proc. nat. Acad. Sci.* **15,** 876–881 (1929).
Svetozarova, V. V., *C. R. Acad. Sci. U.R.S.S.* **23,** 473–477 (1939).
Tai, S. E., *J. agric. Ass. China* **120,** 10–55 (1934); *Plant Breed. Abstr.* **8,** 452.
Taylor, J. W., and Quisenberry, K. S., *J. Amer. Soc. Agron.* **27,** 149–153 (1935).
Teller, G. L., and Teller, W. K., *Cereal Chem.* **9,** 560–572 (1932).
Thompson, W. P., *Genetics* **10,** 285–304 (1925).
Trans. roy. Soc. Can. **22,** 335–348 (1928).
J. Genet. **16,** 309 (1931).
Thompson, W. P., Arnason, T. J., and Love, R. M., *Canad. J. Res.* **12,** 335–345 (1935).
Thompson, W. P., and Cameron, D. R., *Genetics* **13,** 456–469 (1928).
Thompson, W. P., and Hutcheson, I., *Canad. J. Res. C* **20,** 267–281 (1942).
Thompson, W. P., and Thompson, M. G., *Cytologia,* Fujii Jubil. Vol., 336–342 (1937).
Tschermak, E. von, Weizen. Bastardierung. In Fruwirth, C., Handbuch der landwirtschaftlichen Pflanzenzüchtung, Bd. 4, 4 Aufl. Berlin (1923). [Cited by Kajanus in *Bibliogr. Genet.* **3,** 141–244 (1927).]
Ber. dtsch. bot. Ges. **47,** 253–261 (1929).
Ber. dtsch. bot. Ges. **48,** 400–407 (1930).
Tschermak, E. von, and Bleier, H., *Ber. dtsch. bot. Ges.* **44,** 110–131 (1926).
Tumanien, M. G., *C. R. Acad. Sci. U.R.S.S.* **16,** 325–327 (1937).
[Toumanian]. *Soviet Bot.* 1938(6), 100–102 (1938); *Plant. Breed. Abstr.* **9,** 1050.
Tzitzin, N. V., *Lenin Acad. Agric. Sci., Siberian Inst. Grain Cult. Omsk* 1933, **101** (1933).
[Cicin]. *Breed. Seed Growing.* 1936(2), 21–27 (1936); *Herb. Abstr.* **7,** 14–15.
Breed. Seed Growing **10,** 4–7 (1940); *Plant Breed. Abstr.* **11,** 942.
Uchikawa, I., *Jap. J. Genet.* **13,** 9–15 (1937).
Jap. J. Genet. **14,** 264–267 (1938).
Umar, S. M., *Indian J. Genet. Plant Breed.* **3,** 61–66 (1943). *Plant Breed. Abstr.* **14,** 706.
Vacenko, A. A., *C. R. Acad. Sci. U.R.S.S.,* N. S. 5, 338–343 (1934).
Vakar [Wakar], B. A., *Züchter* **7,** 199–206 (1935).
Cytologia **7,** 293–312 (1936).
Cytologia **8,** 67–90 (1937).
Veruschkine, S. M., *People's Commissariat Agric. U.S.S.R., Saratov* 1935, pp. 39 (1935); *Plant Breed. Abstr.* **6,** 138.
Breed. Seed Growing. 1936(2), 28–33 (1936a); *Plant Breed. Abstr.* **7,** 595.
J. Bot. U.R.S.S. **21,** 176–185 (1936b); *Plant Breed. Abstr.* **9,** 191.
Voss, J., *Angew. Bot.* **20,** 265–348 (1938). *Plant Breed. Abstr.* **9,** 698.

Wagner, S., *Arch. Klaus-Stift. Vererb.-Forsch.* **17,** 449–453 (1942); *Plant Breed. Abstr.* **14,** 848.

Watkins, A. E., *J. Genet.* **19,** 81–96 (1927).

J. Genet. **20,** 1–27 (1928).

J. Genet. **23,** 173–263 (1930).

J. Genet. **39,** 249–264 (1940).

Watkins, A. E., and Ellerton, S., *J. Genet.* **40,** 243–270 (1940).

White, W. J., *Sci. Agric.* **21,** 198–232 (1940).

Worzella, W. W., *J. agric. Res.* **65,** 501–522 (1942).

Yamasaki, Y., *Jap. J. Genet.* **11,** 314–315 (1935); *Plant Breed. Abstr.* **6,** 842.

Jap. J. Bot. **8,** 151–153 (1936).

Yamashita, K., *Jap. J. Genet.* **13,** 15–17 (1937a).

Mem. Coll. Agric., Kyoto **39,** 9–38 (1937b); *Plant Breed. Abstr.* **8,** 457.

Yefeikin, A. K., and Vasilyev, B. I., *Bull. appl. Bot., Genet., Plant Breed.*, Ser. **2**(9), 39–45 (1936).

Zhebrak, A. R., *C. R. Acad. Sci. U.R.S.S.* **25,** 56–59 (1939).

C. R. Acad. Sci. U.R.S.S. **29,** 400–403 (1940a).

C. R. Acad. Sci. U.R.S.S. **29,** 480–482 (1940b).

C. R. Acad. Sci. U.R.S.S. **31,** 485–487 (1941a).

C. R. Acad. Sci. U.R.S.S. **31,** 617–619 (1941b).

Social. Agric. (8), 51–57 (1943); *Plant Breed. Abstr.* **14,** 1202.

C. R. Acad. Sci. U.R.S.S. **43,** 120–121 (1944a).

Nature, London **153,** 549–551 (1944b).

Zhukovsky, P. M., *Trans. K. A. Timiriaseff Acad. Agric., Moscow* **6,** pp. 48 (1944); *Plant Breed. Abstr.* **15,** 549.

Genetic Effects of Radiations

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CONTENTS

I. INTRODUCTION

Genetic effects have been produced by means of radiations in a wide range of organisms, often only or mainly with the object of obtaining genetic variants for use in various researches, for example, in Neurospora for the study of biosynthesis (Beadle and Tatum, 1945), in Drosophila for the study of position effect (Stern, 1944) and in Zea for the cytological mapping of gene loci (Anderson and Randolph, 1945). In this review we shall be concerned only with the genetic effects of radiations in so far as these throw light on the mechanisms involved or upon the nature of the genetic changes and of the gene itself. The survey is therefore necessarily selective and much literature of little analytical value to our purpose is neglected. Reference to some of it will be found in previous monographs and reviews (Catcheside, 1945; Hanson, 1933; Lea, 1945; Schultz, 1936; Timoféeff-

Ressovsky, 1930, 1934). No detailed consideration will be afforded genetic effects of radiations such as the induction of non-disjunction (Anderson, 1924, 1931), of attached-X detachment (Mainz, 1940) of oogonial and spermatogonial crossing-over in Drosophila (Friesen, 1936; Patterson and Suche, 1934; Whittinghill, 1937), of phenocopies (Blanc and Braun, 1942; Epsteins, 1939) or of position effects (Catcheside, 1947; Dobzhansky, 1936; Dubinin and Sidorov, 1934, 1935; Dubinin *et al*, 1935).

Study of the genetic effects of radiations commenced when Muller (1927) showed in Drosophila that X-rays were able to induce mutations indistinguishable from those that appeared spontaneously. The notable feature of the discovery was that X-rays gave an induced mutation rate many times greater than the natural one. Shortly afterwards (Altenburg, 1928) it was found that X-rays also induced structural changes in the chromosomes, in particular translocations, similar to naturally occurring ones. The induction of genetic changes by X-rays in plants, barley and maize, was announced very shortly afterwards by Stadler (1928a and b). Thus the stage was set and geneticists were not slow to adopt the use of an instrument that would greatly facilitate the production of the genetic tools, gene and chromosome changes, employed in research.

II. The Radiations

The genetically effective radiations are of two kinds, ionizing and non-ionizing. The ionizing radiations are the α-, β- and γ-radiations of radioactive substances, X-rays, protons and neutrons. The only effective non-ionizing radiation is ultraviolet light. The principal means of energy dissipation by an ionizing radiation in its passage through matter is the ejection of electrons from atoms through which it passes. An atom so *ionized* is left positively charged and is referred to as an *ion*. The ions are localised along tracks, the lengths of which in the case of X-rays vary with the initial energies of the quanta and therefore inversely with the wavelength. A quantum in ejecting an electron from an atom passes on some (Compton recoil electron production) or nearly all (photoelectron production) of its energy to the ejected electron. There is no difference in the properties of the recoil and photoelectrons. The ejected electron in its passage through matter causes further ionizations, losing energy with each collision until it finally halts. As the electron loses its energy the ionizations produced become more closely grouped. Thus the ionizations are not scattered uniformly along the whole track but occur in clusters widely spaced in the earlier part and more and more closely spaced in the later part of the track. The tracks of long wavelength (soft) X-rays consist of little more than the densely ionized "tail." γ-rays are natural X-rays of very short wavelength, having quanta of very high energy and producing only Compton recoil electrons. α-rays

are the nuclei of helium atoms, having only a short range in tissue and producing a very large number of ionizations per micron of path. Neutrons mainly project hydrogen nuclei, already in the tissue, at high speed as protons. These produce ionizations intermediate between electrons and α-rays in the density of their distribution along the track.

Dosages of X-rays, γ-rays and α-rays are usually measured in roentgen (r), this unit being the dose of radiation required to liberate 1 electrostatic unit of charge in 0.001293 g. of air. Approximately two ionizations per cubic micron are produced in water or tissue irradiated by a dose of 1 r, the actual figure differing somewhat for different radiations and for different compositions of tissue.

Neutron dosimetry presents certain difficulties, and diverse units are in use (Gray, 1943). One n-unit of neutrons (used in America) produces approximately the same amount of ionization in tissue as 2.5 r of X- or γ-rays. One ν-unit of neutrons (used in some British work) produces approximately the same ionization in tissue as 1 r of X- or γ-rays.

Where it is specially desired to compare the efficiencies of radiations of different types, it is convenient to express all dosages in "energy-units." One energy-unit (E) of any radiation results in the same dissipation of energy in water, namely an amount equal to the dissipation of energy in water by 1 r of γ-rays.

The biological action of radiation is most plausibly attributed to chemical change resulting from the ionization. Since the chemical bonds which hold a molecule together are constituted by electrons shared between the two atoms joined by the bond, the removal of a bonding electron is likely to lead to its dissociation or to other chemical change. The removal of electrons other than bonding ones is also likely to lead to chemical change, since the energy involved in ionization greatly exceeds that required to remove an atom from the molecule.

Ionizing radiations also dissipate energy in tissue by *excitation*, *i.e.* by raising an electron in an atom or molecule to a state of higher energy. This is a less drastic process than the ejection of the electron and is likely to be less efficient in producing changes. Ultraviolet light can cause excitation but not ionization.

For a recent full account of the physical data known about genetically active radiations, reference should be made to Lea (1945).

III. General Cytological Effects of the Radiations

Similar changes are observable in different tissues, *e.g.* root tips and the male gametophyte in the pollen grain and pollen tube. The latter is particularly convenient for study as a more precise control of the division affected is possible.

Irradiation is liable to produce a lengthening of the nuclear division cycle. It has been suggested that this is due to an effect on the nucleic acid cycle, resulting in a failure of the conversion of ribonucleic acid to the desoxyribonucleic acid form. Ribonucleotides accumulate in the cytoplasm after irradiation (Mitchell, 1942). Higher doses inhibit mitosis for a period dependent upon the dose employed. The dose required for this inhibition is inversely proportional to the number of chromosomes (Marshak and Bradley, 1944), but is not correlated, directly or inversely, with chromosome length. This indicates that the inhibition of mitosis by X-rays occurs through an action upon the centromeres.

Another probable effect on the centromeres is the induction of diplochromosomes, with four chromatids instead of two (White, 1935; Carlson, 1941b; Darlington and La Cour, 1945).

Apart from the above changes two kinds of effects on the chromosomes have been observed. One is a *physiological* change, due to an alteration in the surface properties of the chromosomes causing them to stick together (Alberti and Politzer, 1923, 1924; Darlington and La Cour, 1945; Marquardt, 1938; Pekarek, 1927). This appears to be an effect on the nucleic acid also and is believed to be due (Darlington, 1942) to the non-sticky polymerised nucleic acid coating on the condensing and spiralizing chromosome being broken down by the irradiation to a fluid non-polymerised state in which parts on different chromosomes become attached to one another. The stage of the mitotic cycle most sensitive to these physiological effects is in the latter part of the prophase. The other is a *structural* change, due to the production of breaks in the chromosomes followed by the formation of structural rearrangements resulting from the recombination of the breakage ends in various ways.

The physiological effect is shown only in cells which are already in the prophase or later stages of division at the time of irradiation. They continue division, though the various stages are somewhat lengthened. Cells at mid to late prophase at the time of irradiation will show stickiness leading to clumped metaphases and to anaphase bridges even with quite small doses. With large doses excessive clumping may prevent the completion of mitosis. Nuclei at resting or early prophase stages (Carlson, 1941) at the time of irradiation are delayed in division, the extent of the delay depending upon the dose and the material. Such nuclei recover from any physiological effect they may have suffered, for no stickiness appears when they reach the metaphase and anaphase. Instead they may show structural changes. The chromosome breaks which are the primary cause of these seem to be produced almost instantaneously by the radiations. Breaks have been observed at diakinesis within 3 minutes of irradiation (Bishop, 1942), but

usually breaks if present are obscured by stickiness for some hours after irradiation.

IV. Types of Structural Change

Structural changes observed at the metaphase of the division cycle in which they are produced are of two kinds, (a) *chromosome*, where both chromatids are similarly affected and (b) *chromatid*, where only one of the two chromatids is affected at a given place.

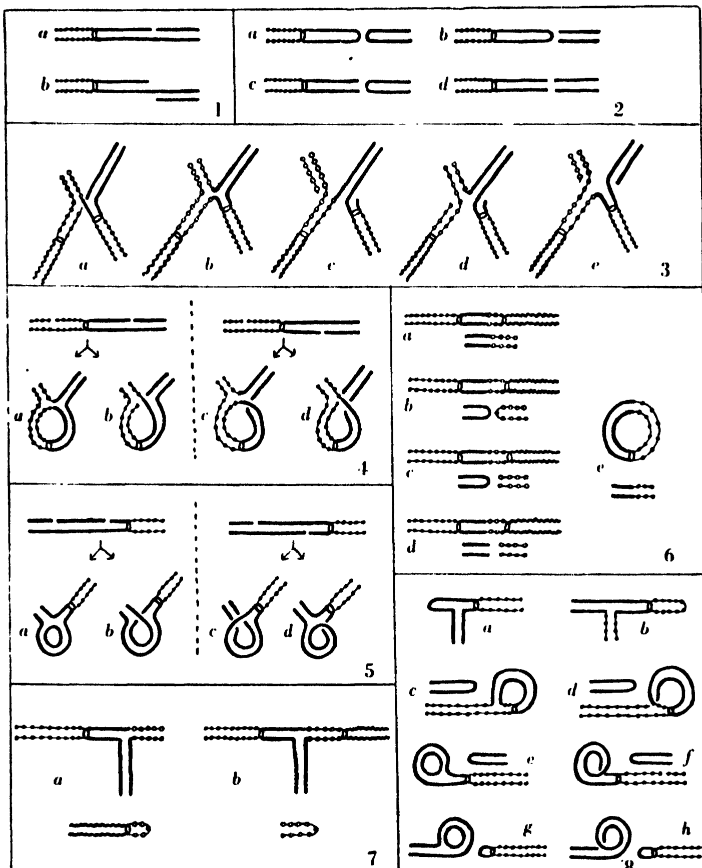
X-ray experiments furnish evidence about the time of chromosome splitting preparatory for mitosis (Mather, 1937; Mather and Stone, 1933; Riley, 1936). This time is clearly prior to prophase, but opinions range from this period to as far back as the anaphase of the previous division but one (Nebel, 1933). The interpretation of the radiation data is deferred to section VII.

The metaphase appearances of the structural changes are here described without prejudice to any hypothesis as to how and when the various types are produced.

(a) A *chromosome break* (= terminal deletion = B″ of Darlington and La Cour (1945)) consists of a fracture of a chromosome into a centric and an acentric fragment (fig. 2). The latter tends to become lost from the daughter nuclei through its lack of a centromere (Carlson, 1938b). The breakage ends produced by the fracture frequently undergo *restitution*, in which case the original structure is restored, or they may unite in new combinations with other breakage ends. The restitutions may be represented by constrictions in metaphase chromosomes (Carlson, 1941b). The surviving chromosome breaks observed at metaphase are only a fraction of the breaks primarily produced (Catcheside, Lea and Thoday, 1945b; Lea, 1945; Lea and Catcheside, 1942).

The chromosome is commonly fractured when it is unsplit. Later, following splitting in the centric and acentric fragments, sister union may occur between sister chromatid breakage ends in either or both fragments, to produce a dicentric chromatid and/or an acentric U-shaped fragment. The appearance of the products of a chromosome break therefore varies (fig. 2). The sister chromatids may undergo union at the breakage ends (SU = sister chromatid union), or sister union may fail at one (NUp = proximal non-union of sister chromatids; NUd = distal non-union) or both (NUpd = proximal and distal non-union) of the broken ends of the two fragments. Darlington and La Cour (1945) score a sister-union as C_1SR or C_0SR, according to whether it has occurred in a centric or an acentric fragment. Sister union is more frequent in acentric fragments at all stages of the nuclear cycle (*ibid*). It is interpreted by some (Darlington and Upcott,

1941) as a reproductive error of a gene and not a true reunion. Not only is there the problem of how sister union takes place, but also that of why it fails to occur when the breaks are produced at certain stages of the nuclear cycle by ionizing radiations, for in the case of breaks mechanically produced



FIGS. 1–8

Diagrams of chromosome structural changes induced by radiations. (1) Chromatid breaks; (2) chromosome or isochromatid breaks; (3) Chromatid interchanges, c, d and e being incomplete; (4) inter-arm chromatid exchanges; (5) intra-arm chromatid exchanges; (6) asymmetrical chromosome exchanges; (7, 8) chromatid-isochromatid exchanges. (After Catcheside, Lea and Thoday, 1945b.)

at telophase sister union occurs almost always (McClintock, 1941a), though even these breaks may "heal" against sister union (McClintock, 1941b, 1942a).

At anaphase the dicentric chromatid produced by sister union in the centric fragment will be stretched as a bridge between the two spindle poles and it may break or more likely be cut by wall formation. Each daughter cell would then be left with a chromosome with a broken end. In such cases sister union may occur again when the broken chromosome next divides, leading to further bridge formation and anaphase breakage, as in the male gametophyte and endosperm divisions in maize where bridge formation has followed crossing-over within an inversion (McClintock, 1938a). This may be repeated until loss of the chromosome or death of the cell eventually occurs. Such loss does occur fairly readily, since abnormal mitotic figures disappear almost completely within a few nuclear division cycles after irradiation.

In sporophyte tissues the broken ends normally heal against sister chromatid union (McClintock, 1941b), but if two such ends are together in the same nucleus, they may unite (McClintock, 1942a) to give a dicentric chromosome. A considerable number of chromosome breaks appear in cells with chromatid breaks. There is evidence (Section VII) that the latter, and therefore the former, are produced in chromosomes already divided into two chromatids. Such chromosome breaks with two separately broken chromatids are distinguished by Lea and Catcheside (1942) as isochromatid breaks.

(b) A *chromatid break* (= B′ of Darlington and La Cour, 1945) shows as a break at a given place in one of the two chromatids of a metaphase chromosome. The acentric fragment remains close beside the intact sister chromatid, though not necessarily in line with the centric fragment (fig. 1). As with chromosome breaks, it can be shown that the chromatid breaks observed at metaphase constitute but a minority of those originally produced. The majority restitute or take part in exchange.

(c) An *exchange* (2R′ or 2R″ of Darlington and La Cour, 1945) appears when the four breakage ends produced by two separate breaks rejoin two-by-two in a new way (figs. 3–8). The two breaks concerned may be in the same chromosome or in separate chromosomes. Both breaks in an exchange may be chromatid (figs. 3–5), or both chromosome (fig. 6), or one may be chromatid and the other chromosome (figs. 7, 8; Carlson, 1938a; White, 1935). The breakage ends may be united so that the exchange is symmetrical (*e.g.* fig. 3a) or asymmetrical (*e.g.* fig. 3b). Where reunion of two chromatid breaks in the same chromosome occurs, differences result according to whether the breaks are in the same chromatid or not. Accordingly, the new chromatids may have inversions, duplications or deficiencies and possess one, two or no centromeres. Finally, one of the two possible reunions (R′ or R″ of Darlington and La Cour, 1945), according to whether it is a chromatid or chromosome reunion) may fail, an incom-

plete exchange (figs. 3c, d, e) resulting (Catcheside and Lea, 1945; Catcheside, Lea and Thoday, 1945a). The variety of exchanges theoretically possible is therefore very considerable, and most have been observed (Catcheside, Lea and Thoday, 1945a; Darlington and La Cour, 1945) in Tradescantia and Trillium. Some types, particularly symmetrical chromosome exchanges (not illustrated), give effects that are rarely detectable in somatic chromosomes, so that for quantitative purposes they are better ignored in such material.

In the cells of irradiated pollen grains and roots, exchanges seem to be strictly confined to those occurring between pairs of breaks. In Drosophila, on the contrary, an appreciable proportion of exchanges occur between three or four breaks, the broken ends rejoining in cyclical order (Bauer *et al*, 1938; Catcheside, 1938). This implies a difference in the conditions of rejoining in the two cases, that in Drosophila allowing potentially more kinds of structural change.

Only some of the types of exchange are viable in the sense that they are capable of transmission unchanged to offspring which are capable of normal life. Symmetrical exchange between two chromosomes yields an *interchange*, or translocation. Symmetrical exchange between opposite arms of the same chromosome yields a pericentric inversion (Muller), while asymmetrical exchange within a chromosome arm yields a paracentric inversion (Muller). An intra-arm chromatid intrachange, where the breaks are in separate chromatids, could yield a direct contiguous duplication (Catcheside, 1947) of the type found in Bar. Deficiencies not involving the centromere are often viable when the lost fragment is not too large. Interpolations such as are common in Drosophila appear absent from pollen grain and root material.

Symmetrical and asymmetrical interchanges appear to be about equally frequent (Catcheside *et al*, 1945a; Newcombe, 1942; Swanson, 1942), though according to some reports asymmetrical interchanges are more frequent (Sax, 1940; Sax and Mather, 1939; Swanson, 1942). In the case of intrachanges also, asymmetrical and symmetrical exchanges are equally frequent. These results provide no evidence of any polarization in the chromosome such as would prevent random joining of breakage ends. Muller (1941) has pointed out that this fact renders it improbable that breakages and union occur in linkages in a polypeptide chain, since such linkages are polarized.

(d) A *minute* interstitial deletion is produced by asymmetrical intrachange between breaks produced close together in the same chromosome or chromatid. The justification for distinguishing them formally from other exchanges is that they show a disproportionately high frequency in Drosophila and Tradescantia compared with other exchanges. The difference is

analysed further in Section VI. In pollen grains the deleted portions are observed (Darlington and La Cour, 1945; Giles, 1943; Newcombe, 1942a and b; Rick, 1940) as small bodies (isodiametric fragments) most commonly about 1 μ diameter. In some cases it can be seen, but usually it is presumed, that they are rings. These rings are probably most often chromosome rings, *i.e.* consist of two chromatid rings lying together, but they are usually too small and condensed to show definite structure. It is by no means certain that these minute deletions correspond to the minute deficiencies observed in Drosophila (Hoover, 1938; Slizynski, 1938, 1942), which consist of losses of only one or a few bands (*i.e.* 0.1%–0.5% of the chromosome length) and which may be produced by a different process.

V. Sequence of Induced Structural Change in the Mitotic Cycle

It is found that when metaphases are observed at different times after irradiation (Carlson, 1941b; Darlington and La Cour, 1945) the kinds of structural change and their frequency for a given dose change in characteristic ways (figs. 9—12). For a period after irradiation the metaphases, *i.e.* in those cells irradiated at a stage relatively close to metaphase, show clumping or stickiness, but no breaks. However, irradiation of metaphase chromosomes (at first meiotic division in Sciara) can produce breaks, but not detectable at the division in which they are produced (Reynolds, 1941). In cells reaching metaphase subsequently no clumping appears, but instead breaks and reunions may occur. The frequency per cell of these rises to a maximum and then declines. In Tradescantia pollen grains irradiated at 20°C. and maintained at this temperature the frequency per cell of total chromatid breaks attains a peak about 12 hours later. The peak frequency of chromatid reunions occurs at the same time. In the case of chromosome breaks, two peaks of frequency usually occur. One appears with or more usually somewhat later than the chromatid break peak. The second peak appears at 30–36 hours after irradiation and is a lower one. The peak frequency of chromosome reunions appears with, or somewhat later than, the second total chromosome break peak. On the other hand reunions involving a chromatid and chromosome break appear a considerable time before chromosome-chromosome reunions. They tend to show a peak at the same time as the chromatid reunion peak and may show a secondary peak at the time when purely chromosome reunions first appear and long before the latter show their peak frequency. Sister union at the broken ends of the centric and acentric fragments derived from chromosome breakage also shows characteristic changes. It is at a maximum in cells which reach metaphase relatively soon after irradiation and thereafter declines, more rapidly in centric fragments, from which it has disappeared in cells kept at 20°C.

which reach metaphase 48 hours after irradiation. In acentric fragments the disappearance of sister union is somewhat more gradual and may never be complete (Darlington and La Cour, 1945).

The interpretation is complicated by the fact that different cells, in a root or an anther, develop at different rates. In roots (Darlington and

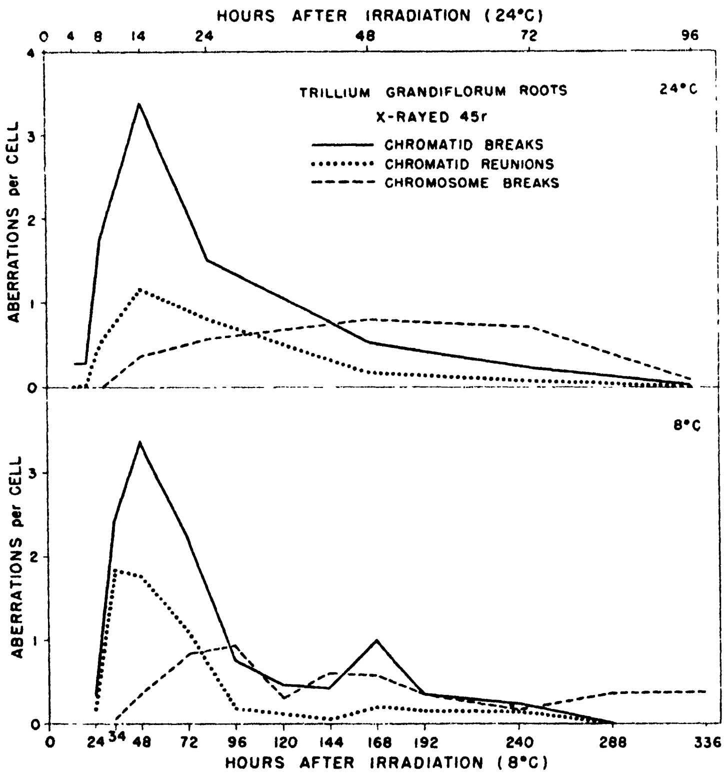


FIG. 9

Temporal sequence of breaks and reunions in *Trillium grandiflorum* roots. (Data of Darlington and La Cour, 1945.)

La Cour, 1945) the different rates of development cause continued divergent development of cells so that different phases overlap, and the overlapping is accentuated by low temperature and by higher doses of X-rays. Pollen grains show convergent development, with the latest dividing cells reaching metaphase relatively more quickly than the earlier ones, producing an overlap in a different way.

Most experimenters, investigating the effect of variables such as dosage, intensity and temperature on breakage and reunion, have used fixed

times after irradiation for making quantitative comparisons. It is obviously difficult to interpret the results completely until it is known whether and in what ways the physical variables affect the temporal variation in frequency of the quantities being measured. Darlington and La Cour (1945) have given a good deal of information and Lea and Catcheside have unpub-

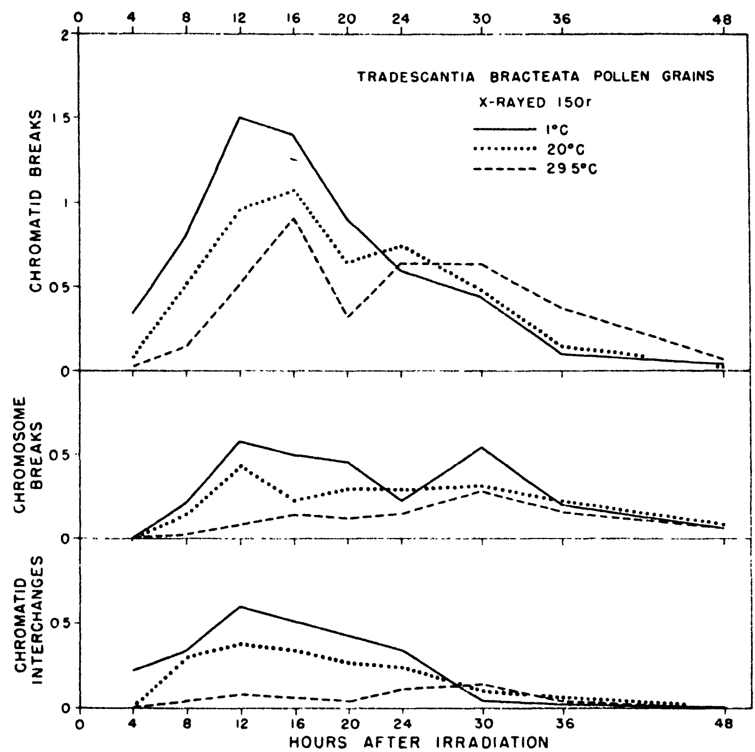


Fig. 10

Effect of temperature at the time of irradiation on the temporal sequence of breaks and exchanges. (Catcheside and Lea, unpublished.)

lished data bearing upon specific points. The following attempts to summarize the results, but it must be realised that the inferred rules are tentative and that much more information is needed before a completely satisfactory account can be given.

Different clones of the same species of plant show more or less marked differences in susceptibility to breakage and reunion. Except where specifically stated, the following effects of external variables on the temporal sequence are those shown by roots or pollen-grains of the same clone. Admittedly the pollen grains of a clonal plant must be a mixed population

genetically, but the chief effect of this must be to mask some of the less sharply defined alterations due to an external variable.

At low doses of X-rays the temporal positions of the peaks are not altered, though somewhat higher doses seem to induce a slightly earlier

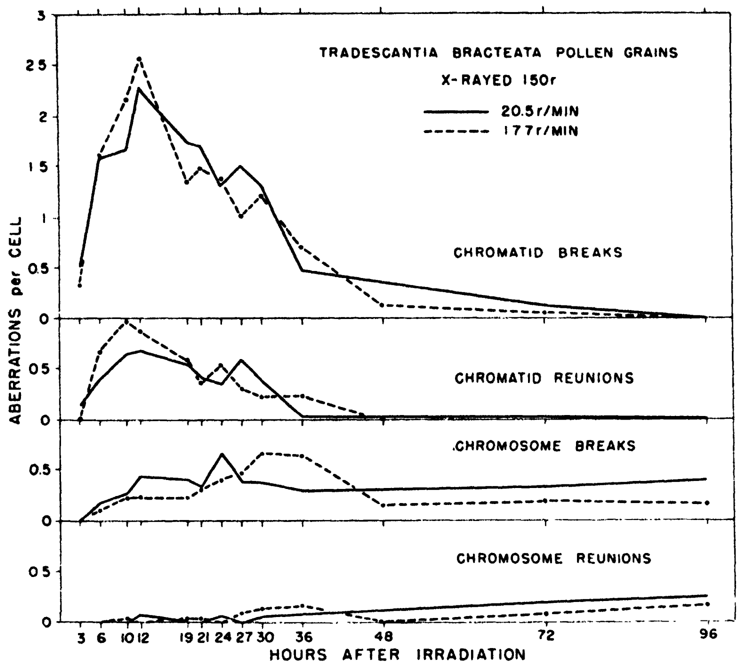


FIG. 11

Effect of intensity of irradiation on the temporal sequence of breaks and exchanges. (Catcheside and Lea, unpublished.)

appearance of them. Rather high doses, which result in suppression of mitosis for a period beginning a few hours after irradiation, cause a fairly considerable delay in the appearance of the peak frequencies, as in Allium roots (Darlington and La Cour, 1945).

Irradiation at a low temperature and maintenance of the material at the low temperature during the period of development of the cells prolongs the nuclear cycle and results in a delay of the appearance of the various peak frequencies as compared with material treated and maintained at a high temperature. The order of the peaks is unaltered but their separation in time is accentuated. According to Darlington and La Cour (1945), temperature does not affect the frequency of breakage (fig. 9) and they therefore ascribe Sax and Enzmann's (1939) results to a comparison being made between different stages of the nuclear cycle. However in these latter and

still other experiments (Catcheside *et al*, 1945b) the buds were maintained at the same temperature except for short periods around the actual time of irradiation. Experiments designed specifically to test the effect of these particular conditions on the sequence, time of appearance and magnitude of the effects have been made. They show (fig. 10) that when *Tradescantia bracteata* pollen grains are irradiated at different temperatures (1°, 20° and 29.5°C.), kept at these temperatures for an hour afterwards and then all maintained at the same temperature (19.5°C.), the low temperature at the time of irradiation has no effect in delaying the appearance of the peak frequencies of breakage and reunion. However, the frequencies of recovered breakage and of reunion are greater at lower than at higher temperatures.

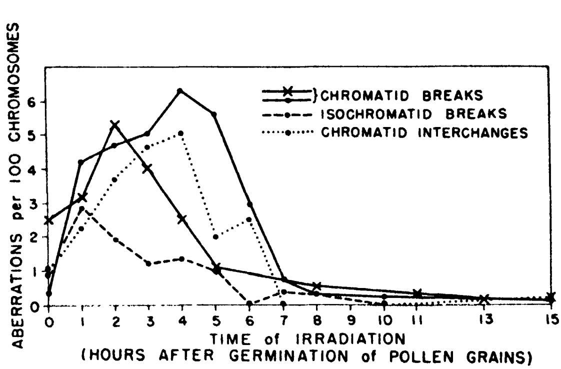


FIG. 12

Yields of breaks and exchanges from irradiation of Tradescantia pollen tubes at different stages of prophase (Swanson, 1943). Crosses ultraviolet, points 370 r X-rays; note: the 0 and 1 hour ultraviolet values are thought to be too low.

This substantiates what has been argued elsewhere (Sax and Enzmann, 1939; Lea and Catcheside, 1942), namely that low temperature at the time of irradiation favours new unions by delaying restitution. Some of Darlington and La Cour's own data (1945, Table 6, p. 208, roots of *Trillium grandiflorum*) show the frequency of chromatid reunion to be higher at 8°C. than at 24°C. Recent unpublished results indicate that the temperature at the time of irradiation controls the yield of breaks very critically, whereas the temperature at other times is without much significance except for its effect upon the rate of development. Irradiation with the same dose at different intensities (fig. 11) causes no change in the time of appearance of the peak frequencies. The amount of reunion is lower at lower intensities, but the frequencies of residual chromatid and of residual chromosome breaks are not different at different intensities. This accords with the view (Lea and Catcheside, 1942; Sax, 1939, 1940) that high intensity promotes new

reunions by providing more contemporaneous breaks in a nucleus than does a low intensity, during the exposure to which some of the earlier breaks will have restituted before the later ones are formed.

VI. Quantitative Relations

(a) *Relative frequency of inter- and intrachanges.* An exchange can occur only if the breakage ends have opportunity to come into contact. Thus spatial considerations may affect the chance. If the observed relative frequencies of intra-arm and inter-arm intrachanges and of interchanges are compared with the frequencies calculated on the assumption that union between breaks is random, the following conclusions may be drawn: (a) There is no marked preference for intrachanges in the same arm as compared with those between opposite arms, or *vice versa*, in Drosophila sperm (Catcheside, 1938a) or Tradescantia pollen grains and pollen tubes (Catcheside *et al*, 1945a; Newcombe, 1942a). If however minute interstitial deletions are taken into account, the proportion of asymmetrical chromosome intrachanges which have both breaks in the same arm in *Tradescantia* pollen grains is 0.84 (Rick, 1940). (b) The proportion of exchanges in which both breaks are in one chromosome, rather than in separate chromosomes, is higher than would be expected on the basis of random union between breakage ends. This holds for Drosophila sperm (Catcheside, 1938; Helfer, 1940; Koller and Ahmed, 1942), Allium root (Sax, 1941), Tulipa pollen grains (Newcombe, 1942a), Tradescantia pollen grains (Catcheside *et al*, 1945a; Newcombe, 1942a; Sax, 1940; Sax and Enzmann, 1939; Thoday, 1942) and pollen tubes (Newcombe, 1942a; Catcheside *et al*, 1943). When unfertilized eggs of Sciara (Metz and Bozeman, 1940; Bozeman, 1943) or of Drosophila (Glass, 1940) are irradiated, the preference for intrachanges becomes extreme, the proportion of interchanges being very small.

(b) *Location of breaks in chromosomes.* It is important to determine whether breaks are produced at random in the chromosomes, or are specially liable to occur at certain points. In *Drosophila melanogaster* the breaks can be very precisely located by observation of the salivary chromosomes, though the observation is then limited to those breaks which have taken part in viable types of structural change, particularly inversions and symmetrical interchanges. The evidence suggests that breaks are randomly distributed along the chromosome and that, if mitotic length is considered, euchromatic and heterochromatic regions are equally breakable (Kaufmann, 1939, 1941b; Kaufmann and Demerec, 1937; Bauer *et al*, 1938). On the contrary, Darlington and La Cour (1945) give evidence that in Trillium roots, the heterochromatin is unbreakable, at least when it is charged with thymonucleic acid.

Less detailed location is possible in other organisms, *e.g.* in Tradescantia the relative numbers of breaks induced in successive fifths of a chromosome arm have been determined (Sax and Mather, 1939). There is a tendency for more breaks to occur near the centromere than near the free end in pollen grains; the same distribution holds (Sax, 1938) for simple breaks and for exchange breaks. In pollen tubes, the breaks are more frequent near the free ends than near the centromere (Swanson, 1942).

Sax (1942b) compared the frequency of production of chromatid breaks in centric chromosomes with that in acentric fragments and found the frequency per unit length in the fragments was only a tenth that in the centric chromosomes. He used two doses of radiation separated by three days; the first gave chromosome breaks and produced acentric fragments, the second gave the chromatid breaks whose distribution was studied. It is improbable that a real difference in breakability exists, but rather that the effect is the consequence of a difference in rejoinability, restitution being more frequent in acentric than centric fragments.

(c) *Distribution amongst nuclei.* If a single chromosome break is produced by direct action of a single ionizing particle on a chromosome and is unaffected by the presence or absence of other breaks in the nucleus, the relative frequency with which nuclei are found to contain different numbers of aberrations should be distributed according to the Poisson formula, *i.e.* $e^{-m} \cdot m^r/r!$ is the proportion of nuclei with r aberrations if m is the mean number. This is borne out by data for chromatid, isochromatid and chromatid exchange breaks produced by X-rays (Catcheside *et al*, 1945a) and by neutrons (Thoday, unpublished) in Tradescantia pollen grains, for chromosome minute deletions and chromosome asymmetrical exchanges in Tradescantia pollen grains (Rick, 1940) and for breaks produced in grasshopper neuroblast chromosomes (Carlson, 1941a and b) as well as for total chromatid and chromosome breaks (Darlington and La Cour, 1945).

(d) *Restitution.* The frequencies of breaks observed in individual cells gives some information about the extent to which the original breaks survive. It has been shown that the numbers per cell of chromosome and chromatid breaks surviving as such and several types of exchanges involving pairs of united breaks are independently distributed at random to the cells (Section VIe). There can be little doubt that, for example, chromatid breaks and chromatid exchanges represent different ultimate fates of originally the same event, namely a chromatid break. If the original breaks depended upon individual radiation hits distributed amongst the nuclei at random and if all the original breaks were represented as some recognisable aberration in the nuclei, it is clear that chromatid exchanges which absorb the original chromatid breaks predominantly in pairs would affect the distribution of surviving chromatid breaks. However, amongst those with

exchanges the frequencies of cells with even numbers of surviving chromatid breaks per cell show no appreciable deficiency compared with the cells with odd numbers of chromatid breaks per cell.

The effect is also sometimes disclosed, as in Tradescantia (Darlington and La Cour, 1945), by a deficiency of cells with odd-numbered totals of chromatid breaks (simple and reunion) per cell. This symptom is however obscured where a larger proportion of the surviving chromatid breaks appear as actual breaks, namely where what Darlington and La Cour (1945) have termed the empirical coefficient of reunion (ECR′) is lower.

It follows that a proportion, and it must be a substantial proportion, of the original chromatid breaks have undergone a change that has rendered them invisible, by restitution of the original unbroken structure. Failure of restitution permits new union, the two processes competing with one another. The intensity effect on the yield of exchanges (Section VIf) shows that these two processes are spread over an interval of time, commencing with the initiation of breakage by the first hits of an exposure to irradiation. The estimation of the mean life of an original break presents no particular theoretical difficulty, but the known values, the derivation of which are discussed in Section 8, are only approximate for irradiation at certain investigated periods of the nuclear cycle. It is significant, however, that the values derived for two widely separated states of the nuclear cycle are of closely similar orders of magnitude. It may be concluded that the speeds of reunion and therefore of restitution do not alter much over a great part of the nuclear cycle, and that the internal controlling conditions are relatively constant.

(e) *Modifying factors.* The probability of restitution of a primary break is affected by its position in the chromosome (see Section VIb) and by the presence or absence of a centromere, chromatid restitution being more probably in acentric fragments (Sax, 1942b). It is also affected by external agencies. Centrifugation (Sax, 1943) during radiation increases the yield of breaks and of exchanges, presumably through the extra stresses tending to separate the breakage ends at a newly formed break and thus to reduce the chance of restitution.

Conversely, colchicine reduces the yield of chromatid aberrations in Allium roots (Brumfield, 1943). It is possible that this is due to the colchicine reducing movement of the prophase chromosomes and so favouring restitution compared with rearrangement.

Temperature influences the yield of all kinds of aberrations induced by a given dose of radiations though not all reports (Darlington and La Cour, 1945) support this. Where flower buds had remained at the given temperature during the irradiation and for one hour afterwards the yield was lower at high than at lower temperatures (Catcheside *et al*, 1945b; Fabergé,

1940a; Rick, 1940; Sax and Brumfield, 1943; Sax and Enzmann, 1939; see Section V). If temperature affects joining of breakage ends rather than the primary production of breaks it should be possible to reduce the yield of structural changes by raising the temperature during the rejoining process. Sax and Enzmann (1939) irradiated flower buds with 160*r* at 3°C. for 2 minutes and 2 minutes later half of them were transferred to 38° for one hour. Afterwards all were kept at 20°C. until fixation 5 days later. The yield of aberrations was lower in the 3° → 38° batch than in the control set kept at 3°C. the whole time, though not so low as in the batch which were at 38° during the irradiation as well as for an hour afterwards. The result indicates that some joining takes place during the irradiation and that it continues for some time afterwards. It is improbable that the results can be explained, as Darlington and La Cour (1945) have suggested, as due to the lengthening of the nuclear cycle by the relatively short exposure to the low temperature (see Section V). Confirmatory experiments are reported by Sax (1947).

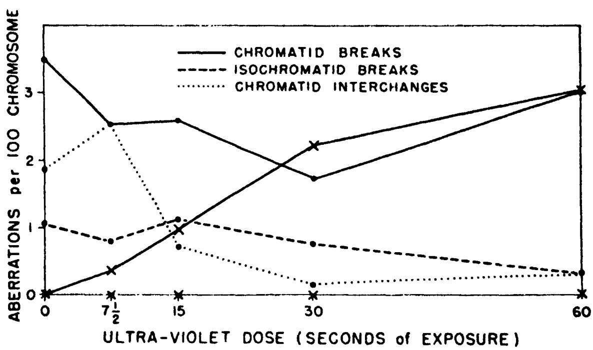


FIG. 13

Effect of ultraviolet in inhibiting X-ray induced structural changes in Tradescantia pollen tubes (Swanson, 1944). Crosses ultraviolet dose alone, points ultraviolet dose followed after 1 hour by 247 r dose of X-rays.

Pre- and post-treatment with ultraviolet light inhibits all kinds of X-ray induced breaks (Swanson, 1944), the degree of inhibition depending upon the dose of ultraviolet used (fig. 13), though the proportionality involved is unknown. Isochromatid breaks are not inhibited by ultraviolet applied after or immediately before X-raying suggesting that they are completely formed immediately upon irradiation. Chromatid interchanges are strongly inhibited if post-treatment is given immediately after X-raying, slightly inhibited if given 30 minutes afterwards and not inhibited and possibly stimulated if given 60 minutes afterwards. Chromatid breaks, however, are still inhibited even when post-treatment is delayed for 60

minutes, suggesting that the ultraviolet promotes restitution. The alteration in the chromosome, by which this effect is brought about, will, when induced prior to X-radiation, have the effect of diminishing effective breakage by promoting restitution.

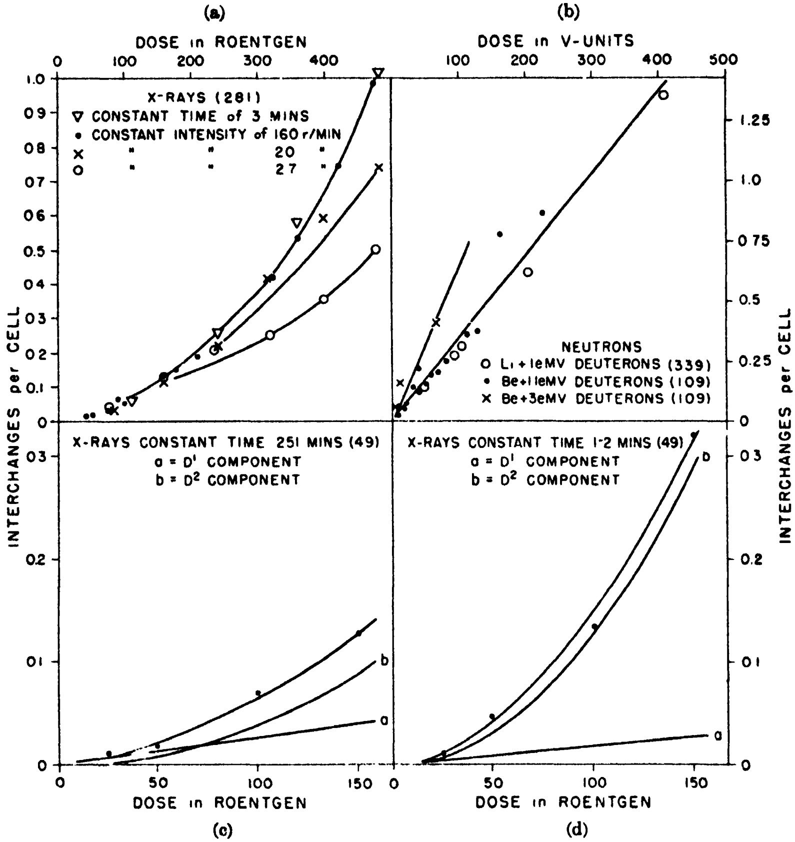


FIG. 14

Effect of dosage of X-rays at different intensities and of neutrons upon the yield of exchanges, in Tradescantia pollen grains.

Near infra red radiation (below 20,000 A) increases the yields of chromatid, isochromatid and chromatid exchanges induced in Tradescantia pollen grains by X-rays (Swanson, 1944). All types of aberration are increased by pretreatment with infra red radiation, which therefore increases actual breakage. A delay as long as 21 hours between infra red and X-ray treatment does not appreciably change the magnitude of the effect, which therefore is of a relatively permanent nature. Post-treatment with infra red increases simple chromatid breaks and chromatid exchanges, but

not isochromatid breaks, which are therefore completely determined immediately on irradiation. This confirms the evidence from combined ultraviolet and X-ray treatment.

The yield is also affected by the state of the chromosomes at the time of irradiation (Section V). In experiments of Swanson (1943) pollen grains were germinated on an artificial medium and irradiated at various times after germination, representing various stages of prophase of the pollen tube mitosis. The maximum for chromatid breaks was at four hours after germination when the nucleus was in mid-prophase and the chromosomes spiralizing; the maximum for isochromatid breaks, however, came earlier (fig. 5). The breakability of the chromosomes in pollen grains of tetraploid species of Tradescantia is only about half that in diploid species (Sax and Swanson, 1941). The cells of dormant bulbs of *Allium cepa* are more sensitive than dry seeds (Sax, 1941a). The aging of seeds (*ibid;* Nichols, 1942) and increase of their water content (Nichols, 1942) following irradiation both result in greater numbers of chromosome aberrations. Pretreatment of tissues with CO_2 (Sizova, 1936) or with ammonia (Sizova, 1936; Marshak, 1938a and b), which alone produce no structural changes, renders them more sensitive to irradiation. Anaerobiosis reduces the frequency of structural changes (Thoday and Read, 1947). The interpretation of these effects is difficult until a more detailed analysis has been made and the whole cycle followed.

(f) *Dependence of yield on radiation intensity.* Experiments with Tradescantia (Sax, 1939, 1940; Giles and Nebel, 1942; Marinelli *et al*, 1942; Giles, 1943; Catcheside *et al*, 1945b) show the manner in which the yield depends upon the time over which a given dose is spread (fig. 15). The yields of chromatid and of isochromatid breaks are independent of intensity (dose-rate) of X-rays and of neutrons. This result is expected on the view that both these aberrations are produced by the passage of a single ionizing particle respectively through one or both of the chromatids of a split chromosome.

The yields of chromatid and of chromosome interchanges produced by a given X-ray dose diminish with increase of the time over which the irradiation is spread, *i.e.* with decreasing intensity. This is readily explicable if the two breaks are produced by separate ionizing particles. If the radiation is extended over a prolonged time, a break in one chromosome has time to restitute before another break with which exchange is possible is produced in its vicinity. Similar decreased yields are found with fractionated doses, with rest periods intervening between the exposures (Sax, 1939, 1941b; Fabergé, 1940a).

With neutrons, however, the yields of exchanges (chromatid or chromo-

some) are independent of the time over which a given dose is spread, suggesting that a single ionizing particle usually causes both the breaks in the neutron induced exchanges (Giles, 1943).

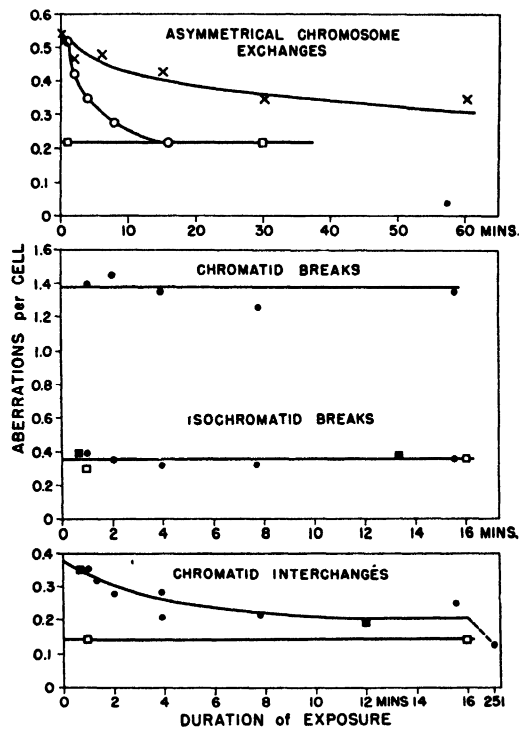


FIG. 15

Effect of intensity of radiation at a given dose upon the yield of exchanges, chromatid and chromosome breaks in Tradescantia pollen grains.

(g) *Dependence of yield on dose.* Simple breaks, namely chromosome or chromatid breaks and isochromatid breaks, increase in linear proportion with increase in dose for X-rays (Sax, 1940, 1941b; Reynolds, 1941; Carlson, 1941a; Thoday, 1942; Newcombe, 1942b; Marinelli *et al*, 1942), for neutrons (Giles, 1940, 1943; Thoday, 1942) and for α-rays (Kotval and Gray, 1947). There appears to be no threshold dose below which breaks do not occur.

Structural changes involving exchanges between two or more breaks produced by X-rays and γ-rays increase more rapidly than the first power of the dose. In earlier experiments with Tradescantia (Sax, 1938) when the dose was varied by varying the duration of exposure at constant intensity, the yield of exchanges increased approximately as the 3/2 power of the dose. Since the yield produced by a given dose diminishes as the duration

of irradiation is extended (Section VIf) the shape of the dose effect curve obtained by varying the duration of exposure at constant intensity depends upon the intensity employed. With high intensities the yield is practically proportional to the square of the dose (Sax and Brumfield, 1943); at lower intensities (fig. 14a) the power of the dose is lowered (Sax, 1941b). A square law is also obtained if the dose is varied by varying the intensity using a constant exposure time (Sax, 1940, 1941b). When different doses are given using intermittent irradiation (Nebel *et al*, 1942), there is a linear dose-effect relation for chromosome exchanges and a more than linear proportionality for minute fragments. It is concluded that fundamentally the yield of X-ray induced exchanges in Tradescantia increases as the square of the dose, and that the breaks involved in an X-ray exchange are separately produced by separate hits, but that the quantitative results are distorted by restitution of breaks unless the irradiation is completed in a *short* time or that the irradiation extends over the *same* time at all doses.

Some experiments (Catcheside *et al*, 1945b) demonstrate that a proportion of X-ray induced exchanges are caused by a single ionizing particle breaking chromatids of two neighbouring chromosomes. The dose curve thus consists of two terms, one proportional to the first power of the dose and the other to the second power of the dose (fig. 14c, d).

With neutrons (Giles, 1940, 1943; Thoday, 1942) and α-rays (Kotval and Gray, 1947) exchanges increase in linear proportion to dose (fig. 14b), a result explicable on the assumption that both the exchange breaks are produced by the same ionizing particle.

(h) *Relative efficiencies of different wavelengths and types of ionizing radiations.* The efficiencies may be compared if X-ray doses are measured in roentgen and neutron doses in ν-units (the American n-unit = 2.5 ν-units approximately), units which represent approximately equal energy dissipations in tissue. The ratio of the yields for equal doses of the two radiations measured in this way represents the ratio of the efficiencies per ionization of the densely ionizing particles (protons) in neutron experiments to the less densely ionizing particles (electrons) in X-ray experiments. These ratios are found to be, for Tradescantia pollen grains:

Chromatid breaks	2.2 Thoday, 1942, Lea and Catcheside, 1942.
Isochromatid breaks	3.6 Thoday, 1942; Lea and Catcheside, 1942.
	4.1 Giles, 1943.
Chromosome breaks	4.3 Thoday, 1942; Lea and Catcheside, 1942.

Values of a similar order of magnitude have been reported for other species of plants (Marshak, 1939, 1942a and b; Marshak and Malloch, 1942). According to Marshak (1942) the ratio of neutron to X-ray efficiency increases by a factor of at least 2 during the nuclear resting stage,

TABLE 1

Coefficients of Aberration Production in Tradescantia (Lea, 1945)

A. Split chromosomes (20–24 hours after radiation, at 20°C.)	*Chromatid breaks per nucleus per r* $10^{-2}\times$	*Isochromatid breaks per nucleus per r* $10^{-2}\times$	*Chromatid exchanges per nucleus* *Per r* $10^{-2}\times$	*Per* r^2 $10^{-5}\times$	*Reference*
X-rays 0.15 A	0.725 ± 0.08	0.271 ± 0.02	....	1.81 ± 0.21	Thoday, 1942; Lea and Catcheside, 1942
1.5 A	0.62 ± 0.12	0.26 ± 0.13	....	....	Catcheside and Lea, 1945
4.1 A	1.10 ± 0.11	0.44 ± 0.11	....	....	Catcheside and Lea, 1945
8.3 A	0.065 ± 0.015	<0.009	....	....	Catcheside and Lea, 1945
Neutrons* (*Li* + *D*)	1.58 ± 0.08	0.99 ± 0.03	0.907 ± 0.03	....	Thoday, 1942; Lea and Catcheside, 1942
α-rays (*Rn* + *RaA* + *RaC*)	1.96 ± 0.09	2.10 ± 0.09	0.59 ± 0.05	....	Kotval and Gray, 1947

B. Unsplit chromosomes (5 days after radiation, at 20°C.)	*Chromosome breaks per nucleus per r* $10^{-2}\times$	*Minute interstitial deletions per nucleus* *Per r* $10^{-2}\times$	*Per* r^2 $10^{-5}\times$	*Asymmetrical exchanges per nucleus* *Per r* $10^{-2}\times$	*Per* r^2 $10^{-5}\times$	*Reference*
X-rays 0.15 A	0.06 ± 0.01	....	....	....	0.52 ± 0.08	Thoday, 1942; Lea and Catcheside, 1942
0.1–1 A	....	....	0.40 ±	....	....	Marinelli, *et al*, 1942; Newcombe, 1942b; Rick, 1940
Neutrons* (*Li* + *D*)	0.26 ± 0.02	....	....	0.32 ± 0.02	....	Thoday, 1942; Lea and Catcheside, 1942
(*Be* + *D*)	....	0.49 ± 0.03	....	....	....	Giles, 1943

* Neutron doses in *v*-units.

indicating a change in the conditions of chromosome response during the resting stage.

A precise figure for the ratio of efficiencies can be stated only when the dosage curves compared are of the same shape, otherwise the ratio would vary with the dose. Kotval and Gray (1947) have found the yields of chromatid and isochromatid breaks to be greater with α-rays than with neutrons, while the yield of chromatid exchanges is lower with α-rays than with neutrons. The precise interpretation of the differences is difficult until more is known about the time progress curves with α-rays and neutrons. However it appears that breakage ends produced in Tradescantia by α-rays are more frequently unjoinable than when produced by X-rays or neutrons.

The relative efficiencies of X-rays (0.15 A) and γ-rays (0.015 A) are not significantly different (Catcheside *et al*, 1945b; Kotval unpub.). Similarly, Fabergé (1940b) found no difference in yield of extra chromosome bodies produced by X-rays of effective wavelengths 0.077 A and 0.44 A. In this region of the X-ray spectrum, however, a considerable change in wavelength produces no marked change in the ion density of the electron paths traversing the material. At still longer wavelengths, an increase in breakability with increase in wavelength has been found (Catcheside and Lea, 1943), a maximum efficiency appearing at about 4 A; the still longer wavelength of 8.3 A was very inefficient.

The known coefficients of production of the various types of aberration are collected in Table 1; they are available only for a few stages of the cell cycle (Lea, 1945). In the case of aberrations the yield of which is proportional to dose and independent of intensity the coefficients given are the values of m obtained by fitting $y = mx$ to the experimental results by the least squares method, y being the number of aberrations and x the dose in r or ν-units. In the case of X-ray induced exchanges, in which the yield from a given dose diminishes with decreasing X-ray intensity, the yields were first extrapolated to zero exposure time (Section VIII) and the extrapolated yields fitted to $y = kx^2$.

(i) *Structural changes induced by ultraviolet light.* Swanson (1940, 1942, 1943) has found that in Tradescantia pollen-tube chromosomes, ultraviolet induces only chromatid breaks, with a frequency directly proportional to dosage. Isochromatid breaks and chromatid exchanges were no more frequent than in control material. Ultraviolet has an effect in promoting restitution as is shown by its inhibitory effect on X-ray breakage (Swanson, 1944, and Section VIe). Maximum sensitivity of Tradescantia prophase pollen-tube chromosomes occurs earlier for ultraviolet than for X-rays (Swanson, 1943).

VII. Time of Split of Chromosomes

The question of when the chromosomes divide, a matter of perennial controversy, was at one time thought to be decided by the change from chromatid to chromosome breakage by X-rays at a critical period in the nuclear cycle. The problem is however confused by the fact that chromosome breaks occur abundantly in the same cells with the chromatid breaks. For this reason it has been suggested that the chromosomes are broken by X-rays only when they are undivided and that they undergo reunions after the chromosomes have divided, yielding either chromosome or chromatid breaks (Darlington and La Cour, 1945; Darlington and Upcott, 1941).

In favour of the chromatid breaks being breaks in divided chromosomes are the following facts, (*i*) The chromatid and chromosome breaks show separate peaks; indeed the chromosome breaks may show two peaks both following irradiation of earlier stages of the nuclear cycle. (*ii*) Exchanges are predominantly between pairs of chromatid or pairs of chromosome breaks depending upon the stage irradiated, chromatid-chromosome exchanges being relatively rare. (*iii*) The frequency of chromatid exchange is closely correlated with the frequency of chromatid breakage. (*iv*) The frequency of sister union bears no relation to the total of chromatid and chromosome breaks, as it would if the chromatid breaks were chromosome breaks in which restitution (or its equivalent) occurred in one chromatid after chromosome division. (*v*) Chromatid breaks appear in *Trillium grandiflorum* pollen, irradiated shortly before metaphase, in cells in which sister unions appear in nearly all chromosome fragments, centric or acentric, which have not taken part in exchanges (Darlington and La Cour, 1945).

In favour of the absence of true chromatid breaks is (*i*) the complete overlap of chromatid and chromosome breaks in roots and pollen grains irradiated at a low temperature, the disproportionate continuance of chromatid breaks in which could be a consequence of delayed reunion. (*ii*) Chromatid and chromosome breaks occur together in many cells: however there is no correlation between the numbers of these breaks in individual cells within single Trillium anthers or roots (Darlington and La Cour, 1945) or in Tradescantia anthers (Catcheside *et al*, 1945a); nor is there any correlation between the numbers of rejoined and unrejoined breaks per cell.

It must be concluded that a proportion of chromatid breaks may be derived from chromosome breaks, but that most are genuine. The average time of splitting should be later than half-time between the peaks of chromatid and chromosome breakage. The time of reproduction may be at the place in development where there is low breakability between these two periods of maximum breakability, both of which lie in the latter half of the resting stage.

In the part of the cell cycle where genuine chromatid breaks are pro-

duced, the apparent chromosome breaks are produced by breakage of each of the two separate chromatids of the chromosome. These are distinguished by their origin from genuine chromosome breaks, *i.e.* breaks of undivided chromosomes, and are called isochromatid breaks (Lea and Catcheside, 1942). The isochromatid breaks which have not taken part in reunion, like the chromatid breaks which have not restituted or reunited in new ways are linearly proportional in frequency to the dose of radiation (though there may be a small dose-square component, Catcheside *et al*, 1945b) and independent of the intensity of radiation at any given dose. Hence each type of break, chromatid and isochromatid, is dependent upon a single hit of an ionizing particle upon an already divided chromosome. An isochromatid break is observed when both chromatids have been hit and broken and no restitution has occurred. A chromatid break is observed when only one of the two chromatids has been broken or when both have been broken and one has restituted. Of these two methods of origin of a chromatid break the former is much the commoner. The evidence for this is that chromatid-isochromatid reunions are much rarer than chromatid-chromatid reunions while isochromatid breaks are not much less common than chromatid breaks.

VIII. Mechanism of Induction of Chromosome Structural Changes

(a) *Direct Action Breakage*

There is no need to recapitulate the whole history of the theory surrounding this subject or to show why the contact hypothesis collapsed in favour of the breakage-first hypothesis (Muller, 1938b). The foundation of a theory was laid by Sax (1938, 1940, 1941b) and attempts (Lea and Catcheside, 1942; Marinelli *et al*, 1942; Fano and Marinelli, 1943) have been made to make it quantitative. Apparently all authors are agreed in assuming that breakage is immediate and simultaneous with the passage through a chromosome of an ionizing particle, though it is not necessary that all "hits" should produce breaks of even a temporary character. This is supported by the absence of a threshold dose for breakage and by the fact that previous irradiation does not render chromosomes more liable to breakage by a later irradiation (Sax, 1942a). If the assumption is correct, the number of breaks produced by a given type of radiation should be proportional to the dose and independent of the intensity and of the temperature. However, not all the breaks primarily produced can be observed, for a considerable proportion restitute (see Section VId). The observable simple breaks are the residue left after some have taken part in exchanges and others have restituted. Since the proportions of breaks that restitute or exchange may depend on intensity and temperature, the tests of whether

the residue is independent of dose, intensity and temperature respectively are unsatisfactory. Indeed a higher temperature during and immediately following radiation results in a smaller yield of chromatid breaks (see Sections 5 and 6e) which could be due to promotion of restitution.

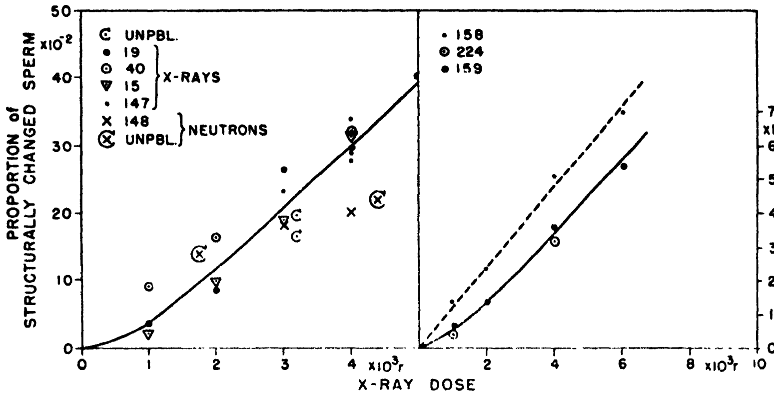


FIG. 16

Relation of proportion of structurally changed sperm to dose of irradiation in Drosophila: (a) cytological determinations in F_1 larval salivary glands; (b) genetical determinations by cubitus interruptus position-effect method.

Exchange and restitution take place during and immediately following irradiation and the yield of X-ray induced exchanges falls with increased duration of exposure. It might therefore be expected that the yield of simple breaks, which constitute the detectable residue, would increase somewhat with increased exposure time. However, if the proportion of the primary breaks that take part in exchange is small, any increased survival of breaks would be slight. Within the accuracy of the experiments, the observed yield of simple breaks is independent of intensity. Thus the intensity experiments and likewise the dosage experiments (Section VIg) are consistent with the yield of primary breaks being independent of intensity. However, the processes of rearrangement and of restitution which intervene between the production of breaks and their observation seriously weaken the value of these experiments as evidence that single breaks are produced by single ionizing particles.

Conclusive proof is afforded by the fact that the yield of exchanges, which in X-ray experiments increases more rapidly than the first power of the dose and is dependent on intensity, in neutron experiments increases linearly with the dose and is independent of intensity (Section VIg). With the doses commonly employed, a pollen grain nucleus is traversed by a score or so of protons in neutron experiments or by a few hundred electrons

in X-ray experiments (Section VIIIb). The theory is that a particular break is produced by a particular one of these particles, not by a cumulative effect due to them all. If the yield of primary breaks is on the average proportional to dose and subject to Poisson distribution in a given nucleus, it is expected that the probability of a pair of breaks being independently produced within a given short distance of one another would be proportional to the square of the dose. The fact that with neutrons the yield of exchanges is proportional to dose shows that the two breaks concerned are not independently produced and is explicable only on the view that the same ionizing particle causes both breaks. The absence of dependence of yield on intensity leads to the same conclusion.

Darlington and La Cour (1945) have formulated a general scheme relating the variations observed in X-ray breakage to the nucleic acid cycle and effects of the radiation upon it. It is difficult to reconcile with the foregoing in all respects. They assume, as do others, that breakage is simultaneous with the irradiation and conclude that it is favoured by the absence of nucleic acid from the chromosome. Thus breakage is reduced to a minimum at prophase by the general attachment of nucleic acid, and at the resting stage is very small in heterochromatin which has a nucleic acid charge.

Sister union is delayed and is simultaneous with chromosome division. Its occurrence is favoured by the absence of a nucleic acid charge on the chromosome or by the charge being non-polymerized, since it is most frequent in those cells with most chromosome breaks. The lower degree of sister union observed in centric fragments is determined by the attachment of nucleic acid being controlled from the centromere. In unirradiated pollen grains with broken chromosomes (McClintock, 1939, 1941a) sister union is normally very frequent, occurring with nearly unit probability. The effect of a dose of radiation is to diminish sister union, the diminution increasing with increasing time between treatment and the time of occurrence of sister union, *i.e.* chromosome division. The radiation accelerates nucleic acid attachment, so healing broken ends against sister union; in acentric fragments the radiation is not able to eliminate sister union completely, though it can do so in centric fragments. Sister union is interpreted as a reproductive error in the end gene next to a chromosome break, rather than a reunion between broken ends following division. This implies that sister union is an indication of chromosome breakage, *i.e.* breakage in undivided chromosomes; apparent chromatid breaks in the same cell would be derived from chromosome breaks.

Chromatid and chromosome reunions (exchanges and restitutions) are delayed and cumulative. Their occurrence is favoured by the presence of a nucleic acid charge on the chromosome. Thus, paradoxically, the condi-

tions that favour breakage, disfavour reunion. For example, chromosome breaks and reunions show a negative correlation in Tradescantia pollen grains; sister union and chromatid reunions a negative correlation in Allium and Trillium roots and Tradescantia pollen grains. Chromatid reunion (the ratio of reunions to total chromatid breaks) decreases with temperature and increases with increasing dose. A higher dose means more breaks, so reunion is favoured against restitution; a higher temperature favours restitution against reunion.

The difficulty of reconciling this theory with all the facts is as follows. If all breaks are originally chromosome breaks, chromatid reunions will have been delayed until after sister union has occurred at the time of chromosome division. If new union is delayed in this way it should be independent of intensity. However, this is not so; therefore chromatid reunions, or the majority of them, occur between chromatid breaks induced in divided chromosomes. Chromosome breaks in the same cells therefore represent breaks of both chromatids of a divided chromosome, and in such isochromatid breaks sister union occurs almost invariably in both centric and acentric fragments.

It is clear that no theory accounts satisfactorily for all the facts and that both the physical and physiological hypotheses have a value in provoking further experiment. In making quantitative investigations of the mechanism of breakage and reunion, some results have been obtained by methods which are outlined in succeeding paragraphs.

(b) *Initial Separation of Exchange Breaks*

The distance apart at the moment of breakage of breaks which later exchange may be deduced independently from three facts, in the employment of which certain physical data are needed (Lea, 1945). Most X-ray experiments have used inhomogeneous radiations of wavelengths 0.1 to 1.0 A. Tissue irradiated by such X-rays is traversed by electrons of a rather wide range of initial energies, thus complicating calculations. Other experiments have shown that there is little or no dependence of yield upon wavelength when the wavelength has been varied between 1.5 and 0.08 A. It is convenient and justifiable to use the physical data for monochromatic X-rays of wavelength 1.5 A even when the experiments were made with shorter wavelengths. All the electrons (photoelectrons) projected in tissue by this wavelength have the same initial energy, 7.5 ekV, and their range in tissue of unit density is 1.5 μ. In each cubic micron of tissue, 6.7 of these photoelectrons are projected by 1000 r. Thus a dose of 50 r produces, on an average, 303 photoelectrons in a nucleus of diameter 12 μ such as that of the Tradescantia pollen grain.

Most of the protons which traverse the tissue in a neutron experiment

have ranges greater than the diameter of the Tradescantia nucleus, so most of them originate outside it. The mean path of a proton in the nucleus is therefore 8 μ (two-thirds of the diameter), and with a dose of 1 ν-unit the total range of all protons in the nucleus is 3.66 μ, being 4.05 μ per 1000 ν-units per cubic micron, in tissue of unit density, for Li + D neutrons. Thus, with a dose of 50 ν-units the Tradescantia nucleus would be traversed on the average by 23 protons.

The fact that the exchanges are predominantly one-hit with neutrons shows that exchanges between breaks are not at random. Even with 23 protons crossing the nucleus the chance of a particular break exchanging with a break due to the same proton, would, were exchange random, be 22 times smaller than the chance of exchanging with a break due to a different proton. It is because the number of ionizing particles traversing the nucleus is small in neutron experiments that, at the low dosages normally employed, the induced exchanges are predominantly *one-hit* (due to the same particle) while in X-ray experiments where the ionizing particles are more numerous they are *two-hit* (due to separate particles).

Suppose a given break may exchange only with a second break produced within a distance h of it. For a two-hit neutron exchange, a second proton would have to pass through a sphere of radius h centred at the given break. Since neutron exchanges at 50 ν-units are not usually two-hit, a second proton usually does not pass through this sphere. Hence the sphere of radius h must have a cross sectional area less than 1/23 of the cross-sectional area of the nucleus; thus $h < 1 \cdot 3\ \mu$.

Since X-ray induced exchanges are usually two-hit with 50 r a second electron is usually found within a radius h of the given break. So the sphere must have a volume exceeding 1/303 of the volume of the nucleus and $h > 0 \cdot 9\ \mu$.

This gives the order of magnitude of h, about 1 μ, based on the simplification that exchange is impossible outside the distance h, whereas probably the chance of exchange diminishes smoothly with increasing separation of the breaks at the moment of their formation.

An independent estimate of h similar in value may be derived from the fact that minute interstitial chromosome deletions and asymmetrical exchanges are about equally frequent. Also the most frequently occurring minute deletion is a sphere about 1 μ diameter, which is also consistent with the distance apart of breaks at the moment of formation being about 1μ.

(c) *Proportion of Breaks That Restitute*

It has been pointed out already that only a proportion, and probably a small proportion, of the primary breaks survive as simple breaks or exchange breaks. Various methods have been proposed to estimate the

proportion of breaks which restitute (Lea and Catcheside, 1942; Lea, 1945; Catcheside, 1945; Catcheside *et al*, 1945b) and hence the number of primary breaks. The methods proposed are: (i) There is a presumed connection between the facts that a proportion of interchanges are incomplete, a proportion of isochromatid breaks do not undergo sister-union and a proportion of chromatid breaks do not restitute, the proportions of the first two being used to estimate the third. (ii) If all breaks took part in exchange, the number of exchanges would be proportional to dose. Since a majority of primary breaks restitute and exchange occurs only between nearby breaks, the yield of two-hit X-ray exchanges increases in proportion to the square of the dose. This dose squared relationship holds strictly only at relatively low doses. At doses where the occurrence of more than two breaks in a critical volume becomes more probable, the yield would increase in a proportion less than the square of the dose, namely be $kD^2\left[1 - \frac{kD^2}{\xi D(1-f)}\right]$, where D = dose, ξ = number of primary breaks, $(1 - f)$ = proportion which restitute or give new unions, and $k = \frac{1}{2}\,\xi^2\,(1-f)^2\frac{h^3}{R^3}$, h being the radius of the volume within which exchange occurs and R the nuclear radius. This expected increase in yield of exchanges in a proportion less than the square of the dose is borne out by some experiments (Newcombe, 1942b) in which high doses have been used. From them approximate limits for ξ and f may be derived. (iii) The yield of two-hit exchanges induced by X-rays is kD^2, where $k = \frac{1}{2}\,\xi^2\,(1-f)\cdot\frac{h^3}{R^3}$. In Tradescantia, R is 6 μ and h is 1 μ, hence ξ and f may be deduced from the experimental values of the coefficient of exchange production. A similar method can be applied to the one-hit exchanges induced by neutrons. (iv) Most interchanges observed in a nucleus X-rayed at a time when the chromosomes were split are chromatid (c/c) exchanges, but a small proportion are interchanges (c/i) between an isochromatid break and a chromatid break. These c/i interchanges are 8.74 times less frequent than the c/c ones at 20 hours after irradiation (Catcheside *et al*, 1945b), hence 17.48 primary chromatid breaks are produced by X-rays to every primary isochromatid break. But the number of chromatid breaks surviving and observed are only 3.843 times as great as the number of isochromatid breaks. Hence f is only $\frac{3.843}{17.48} = 0.22$ times as great as the proportion of primary isochromatid breaks that persist. (v) Estimates of ξ and f may also be derived from consideration of the relative frequencies of chromatid and isochromatid breaks, on the assumption that the production of a

primary chromatid break requires the passage of an ionizing particle through one of the chromatids, and the production of an isochromatid break requires the passage of an ionizing particle through both of the chromatids of a split chromosome.

TABLE 2

Estimates (Catcheside, 1945; Lea, 1945) of the Number of Primary Breaks (ξ) per Cell per Roentgen (or ν-unit), and of the Fraction of Primary Breaks That Persist

	X-rays		Neutrons		α-rays	
	ξ	f	ξ	f	ξ	f
Chromatid breaks	0.09	0.09	0.21	0.09	0.10	0.5
Chromosome breaks	0.07	0.01	0.15	0.02	....	.

(d) *Dependence of Yield of Aberrations on Duration of Exposure and Intensity*

If the dose is administered in a very short time all the primary breaks coexist in the nucleus. If the dose is spread over a longer time, either by use of a low intensity or by fractionating it, many of the breaks restitute before other breaks with which they might exchange are produced in their vicinity. Thus, the yield of exchanges produced by a given dose of X-rays diminishes with increased duration of exposure (Section VIf). If assumptions are made about the time for which a break may remain unrestituted, a theoretical formula can be evolved relating the yield of exchanges to the time of exposure (Lea and Catcheside, 1942, and also Marinelli *et al*, 1942, the formula in which becomes the same when the number of targets is not limited). By fitting such a formula to experimental data, it should be possible to deduce the mean time for which a break remains open before restitution or exchange occurs.

Since the majority of the breaks primarily produced by X-rays in the nucleus restitute, the simplifying assumption may be made that the gradual decrease in the number of open breaks which occurs after the cessation of irradiation is due solely to restitution, that is neglecting the small amount of the decrease that is due to exchange. If n_0 breaks exist in the nucleus at a given moment, the number present after time t in the absence of further irradiation will be $n_0 f(t)$, where $f(t)$ is a diminishing function of t.

The probability of a given break recombining in any short interval of time will be proportional to the number of other breaks within range, *i.e.* the critical distance h. The mean number within range is a fraction

of the total number n existing in the nucleus at that time. The number of breaks combining per unit time is therefore proportional to n^2, say is βn^2. The total number of exchanges formed is $\int \beta n^2 dt = \beta n_0^2 \int \{f(t)\}^2 dt$ (equation I), where the integration extends over the whole period for which breaks exist in the cell.

To proceed further requires a knowledge of $f(t)$. It has been assumed that $n/n_0 = f(t) = e^{-t/\tau}$, $dn/dt = -n/\tau$, implying an average time τ elapsing between breakage and restitution, with the actual times distributed in a skew manner about this mean.

Suppose that while the nucleus is being irradiated at intensity I roentgen per minute, the rate of formation of primary breaks is ξI. Allowing for the rate of reunion, $dn/dt = \xi I - n/\tau$, whence $n = \xi I \tau(1 - e^{-t/\tau})$. At time $t = T$ the radiation stops and then n will decrease according to the equation $n = \xi I \tau(1 - e^{-t/\tau}) \cdot e^{-(t-T)/\tau}$. Substituting in equation (I) and integrating, the estimate of the total number of exchanges is obtained as $\int \beta n^2 dt = \frac{1}{2}\xi^2 \beta \tau (IT)^2 G$ (equation II), where $G = 2(\tau/T)^2 \{T/\tau - 1 + e^{-T/\tau}\}$. Thus the number of exchanges produced in the nucleus by dose $D = IT$ roentgen is proportional to $(\text{dose})^2 \cdot G$. The function G is tabulated by Lea (1945, table 68).

The theory predicts, in accordance with Sax's experiments (1939, 1940), that if the dose is varied by varying the intensity, thus keeping T and G constant, the yield of exchanges is proportional to $(\text{dose})^2$. Secondly, if at constant dose the intensity is varied, the yield of exchanges should be directly proportional to G. By fitting a plot of the G function to the curve of the number of exchanges obtained at constant dose against the time over which the dose is spread, the value of τ may be obtained. In Sax's experiments at room temperature, τ is 4 minutes both for chromatid and chromosome exchanges (Lea and Catcheside, 1942). Various factors appear to influence τ, temperature being one of them (Sax and Enzmann, 1939; Catcheside *et al*, 1945b).

If the dose is varied by varying the time of exposure at constant intensity, G decreases with increase of dose and equation (II) shows that the yield of exchanges would increase less rapidly than the square of the dose. Sax (1938) showed experimentally that the yield of chromosome exchanges obtained with a constant intensity of 25 r per minute increased as the 1.5 power of the dose (Section VI).

The experimental points fit $(\text{dose})^{1 \cdot 5}$ and $(\text{dose})^2 \cdot G$ about equally well. No special significance can be attributed to the 1.5 power in the $(\text{dose})^{1 \cdot 5}$ law that fits these observations. If a lower intensity were used a lower power would have been obtained and if a higher intensity were used a higher power, approximating to 2 at sufficiently high intensities (fig. 14).

So far it has been found that the use of the G function gives a satisfactory account of all experiments having a duration not exceeding 30 minutes exposure. Beyond that limit, the theoretical curve falls below the experimental curve. The explanation may be that $-dn/dt = n/\tau$ does not describe restitution completely, or it may be that a proportion of X-ray exchanges are one-hit. The number of these produced by a given dose would be independent of intensity and exposure time. In experiments designed to detect such one-hit X-ray exchanges (Catcheside *et al*, 1945b) it was found that the dose-yield curves gave clear evidence of a small but constant proportion of one-hit exchanges due to X-rays (fig. 14c, d).

IX. Relative Efficiencies of Different Radiations

By methods described earlier it was possible to deduce from experimental yields of aberrations the numbers (ξ) of chromatid breaks primarily produced per nucleus per roentgen. These are markedly different for X-rays, neutrons and α-rays and in addition show a marked variation with wavelength of X-rays.

If a single ionization could break a chromatid, the efficiency (*i.e.* yield, here ξ) per unit dose would decrease in the order of increasing ion-density, namely in the order X-rays, neutrons, α-rays, since ionizations in the chromatid in excess of the minimum necessary to break it would be wasted. However, neutrons are more efficient than X-rays and it is concluded therefore that a single ionization does not usually cause a break and that a number of ionizations are necessary.

Taking into account the dimensions of the chromatids, some further information may be obtained. The haploid length of the chromosome thread in Tradescantia is 486 μ, and the diameter of the thread about 0.1 μ (Sax and Sax, 1935; Sax, 1938); thus the volume of all 12 chromatids is 7.63 μ^3. Since the total length of α-ray track produced per roentgen per μ^3 is 0.56×10^{3} μ, the total length of α-ray track within the chromatid thread is 4.3×10^{3} μ per roentgen, and the number of intersections of α-rays with the chromatid thread is 0.043 per roentgen. Allowing for δ-rays, the total number of intersections of α-rays and δ-rays with the chromatid thread is 0.113 per roentgen. The number of primary chromatid breaks produced per roentgen is 0.10, indicating that the probability of breakage by an α-particle which traverses the chromatid is almost unity.

Similarly, for neutrons the mean number of chromatid intersections per ν-unit is 0.31, allowing for proton intersections only, or 0.40 allowing also for δ-ray intersections. The number of primary chromatid breaks per ν-unit is 0.21, so the probability of breakage by a proton is somewhat less than unity. Giles' experiments (1943) suggest that the less energetic (more densely ionizing) protons have a higher probability of causing a

breakage than the more energetic (less densely ionizing) ones, in agreement with expectation.

For equal doses of X-rays and neutrons many more chromatids are traversed by ionizing particles in X-ray experiments than in neutron experiments. Yet X-rays produce fewer breaks. Hence the probability of breakage by an electron is rather low, a result that may be ascribed to the ion density in an electron track being on the average much lower than in a proton track. The number of ionizations per micron produced by an electron increases rapidly towards the end of the track, and for the last few tenths of a micron it is of the same order as in a proton track. If this part of an electron track traverses a chromatid, the probability of breakage by it must be quite high. Towards the beginning of an electron track the ion-density is very much lower and it is evident that if this part of the track traverses a chromatid the probability of breakage by it is quite small. It seems probable therefore that an electron is practically ineffective in causing a breakage unless the densely ionizing tail of the trace traverses the chromatid. From the point of view of efficiency per unit dose, all of the track except the tail is largely wasted.

The experimental data for soft X-rays of wavelengths 1.5 A and 4.15 A support this view. These dissipate their energy by means of photoelectrons of 7.5 and 2.5 ekV respectively. For equal numbers of roentgens, the numbers of photoelectrons are in the ratio 1 : 3. The numbers of primary chromatid breaks per roentgen are in the ratio 1 : 1.9. Hence the efficiencies per photoelectron are in the ratio 1.57 : 1.0. The last 2.5 ekV of path of the 7.5 ekV photoelectron (which has a range of 1.5 μ) is indistinguishable from the whole track of the 2.5 ekV photoelectron (of range 0.23 μ). It follows that the probability of a break being caused by the first 1.27 μ of a 7.5 ekV electron is only just over half as great as the probability of a break being caused by the last 0.23 μ.

The length of the effective part of the tail is about 0.28 μ (Lea and Catcheside, 1942). The energy dissipated in a chromatid that intercepts a length of 0.1 μ of the less densely ionized end of the tail is about 0.6 ekV, corresponding to the production of between 15 and 20 ionizations. This is the minimum amount of energy which dissipated in a chromatid is sufficient for the probability of breakage to approach unity. Why such an apparently large amount of energy is needed for chromosome breakage is unknown; nor is it known how the ionizations bring about breakage.

For wavelengths shorter than 1.5 A, there is no appreciable decrease of the number of primary breaks per roentgen with decrease in the wavelength. This is partly accounted for by the occurrence of δ-rays and partly by the less densely ionizing part of the electron track having a low probability of breakage that becomes progressively less negligible compared

with that due to the effective tail as the initial energy of the electron and therefore the length of the track increases.

Although it has been shown that ordinarily a considerable amount of energy from a number of ionizations is required to effect breakage of the chromosome, the sequence of molecular changes by which this is brought about remains unknown.

X. Structural Change in Drosophila

The mechanism of structural rearrangement following irradiation of sperm in Drosophila requires separate consideration if only because cyclic exchanges occur between more than two breaks, leading to complex rearrangements (Dubinin and Khvostova, 1935). Observations can be made only of the changes that are viable at least in the heterozygous state. Consequently all simple breaks and asymmetrical exchanges, which lead to dominant lethals (Section XVII), are absent from the viable offspring that can be studied, whether cytologically in the salivary chromosomes or genetically by means of suitable markers on different chromosomes. Although it is probable that no simple breaks are detected owing to the lethal effect of the loss of the acentric fragment, it is possible that some apparently terminal deficiencies are in fact simple breaks (Demerec, Kaufmann, Sutton and Hinton, 1940; Sutton, 1940). Some data (Demerec, Kaufmann, Sutton and Fano, 1941; Catcheside and Lea, 1945) lead to the conclusion that unrestituted broken ends have a 0.05 chance of healing against sister union.

Intercalary deletions and inversions of the same length appear to be equally frequent (Fano, 1941). Minute deletions, which involve two breaks very close together, are exceptionally abundant in proportion to deletions of larger size (Demerec, 1939a, 1940a; Demerec and Fano, 1941); in the case of the Notch locus deficiencies of less than 15 bands (of the salivary chromosome) constitute a special class, relatively much more frequent than larger deficiencies.

Amongst inversions induced in sperm the proportion with both breaks in the same arm, 0.66 (27 out of 41 inversions) is not significantly different from the expected 0.55 in *D. melanogaster* (Catcheside, 1938a). The proportion of all exchanges that are inversions is considerably greater than expected in *D. melanogaster* (*ibid.*), namely 41 out of 81, or a proportion of 0.506, where 0.36 may be expected on a random distribution; and in *D. pseudoobscura* (Koller and Ahmed, 1942), namely 143 out of 361, or a proportion of 0.396, where 0.20 would be expected (Lea, 1945). This preference for intrachromosomal exchange becomes extreme in the case of irradiated eggs (Glass, 1940), in which the exchanges are almost exclusively inversions.

Breakage frequency is proportional to length within the limits of the euchromatic portion of each salivary chromosome limb (Bauer, *et al*, 1938), but disproportionately high in the heterochromatin, where it occurs with a frequency in proportion to its length in mitotic chromosomes (Kaufmann and Demerec, 1937). Kaufmann (1939) found 23% of all breaks observed in the X-chromosome were in the heterochromatin whence, allowing for inversions entirely within the heterochromatin, the proportion would be 28%. The distribution of 475 breaks in the 114 numbered and lettered segments of the euchromatin of the X-chromosome in Bridge's map is fairly uniform, but the break frequency is exceptionally high in a few segments, for which there is some independent evidence that they contain a little heterochromatin and so are disproportionately small in the salivary chromosome. Similar data are available for *D. pseudoobscura* (Helfer, 1941).

From the available evidence it is therefore possible to state that (i) the probability of breakage is the same for equivalent lengths of each chromosome (ii) the probability of a given breakage end joining to either one of two partner broken ends is equal and (iii) the probability of a given broken end uniting with another break in the same chromosome is about double the probability of it uniting with a break in a separate chromosome. The last generalization is subject to the qualification that breaks very close together in the chromosome, produced by the same ionizing particle, may have an exceptionally high probability of reunion.

It has been mentioned already that all viable structural changes are symmetrical exchanges of some sort, except for the minutes which act as recessive lethals. The simplest kind is an exchange between two breaks (type 2); the only kind with three breaks is that in which cyclic exchange occurs (type 3). With four breaks there may be a cyclic exchange of all four (type 4) or two separate two-break exchanges (type 2 + 2). With six persisting breaks there are four alternatives; 6, 4 + 2, 3 + 3, 2 + 2 + 2 Experimental data (Bauer *et al*, 1938; Bauer, 1939a; Catcheside, 1938a) show considerable discrepancies between observed frequencies of the various types and expected ones, calculated on the assumption of random reunion. Fano (1941) has pointed out that the discrepancies are principally (i) the excessive number of cells with more complicated types of exchange at higher doses (Kaufmann, 1943, reports an extreme case) and (ii) that four-break cases are predominantly type 2 + 2, whereas theoretically type 4 should be twice as frequent as type 2 + 2. The reasons for the discrepancies are unknown, but they indicate that reunions are not random and that two-by-two exchanges are preferred. The expected frequencies, assuming random reunion of breaks, may be calculated by an extension of the theory of dominant lethals (Lea and Catcheside, 1945, see Section

XVII). The proportion of sperm irradiated by dose D in which n breaks are primarily produced and which unite in viable combinations is there found to be $e^{-\alpha D}(2\alpha qD)^n \frac{n!}{2n!}$. Of such sperm a fraction $\frac{1}{2(n-2)!}$ will show type 2 exchange, $\frac{1}{3(n-3)!}$ a type 3, $\frac{1}{4(n-4)!}$ a type 4, $\frac{1}{8(n-4)!}$ a type 2 + 2, and so on.

The yield of symmetrical exchanges is independent of radiation intensity, according to most reports (Catcheside, 1939; Dempster, 1940; Muller, 1940) but not all (Eberhardt, 1939) and independent of fractionation of the dose (Kaufmann, 1941a and c; Muller, 1940) for a given dose in the sperm. Two alternative explanations for this have been suggested: (a) the two breaks taking part in exchange are usually, even with X-rays, produced by the same ionizing particle; (b) no union of breakage ends occurs in sperm, so the breaks accumulate until the opportunity for union occurs during fertilization, *i.e.* when the chromosomes spread out as they enter the egg. The first possibility seems to be ruled out by the experimental evidence (Bauer, 1939b; Bauer *et al*, 1938; Belgovsky, 1937; Khvostova and Gavrilova, 1938; Muller and Altenburg, 1930; Muller, 1940; Timoféeff-Ressovsky, 1939b) that the yield of exchanges increases more rapidly than the first power of the dose. However, some reports of experiments using the *ci* position effect show a linear relation between dose and the yield of exchanges (Eberhardt, 1939; Khvostova and Gavrilova, 1935). It has been suggested (Catcheside, 1938a) that a power of the dose greater than the first could be obtained experimentally, even though the pairs of breaks were caused by the same particle. Since each independent exchange has only a half chance of being symmetrical and therefore potentially viable the proportion of translocation individuals amongst the viable survivors would increase according to the relation, exchanges $= k \cdot e^{-\eta D}$, that is in a proportion greater than the first power of the dose. The same effect would not occur in the case of changes such as recessive lethals, since these are produced independently of any dominant lethal effect.

The second suggestion is apparently supported by cases reported of apparent exchange between a paternal chromosome broken in the sperm by X-rays and a maternal chromosome spontaneously broken (Sidky, 1940). Experiments designed to detect interchange between maternal and paternal chromosomes separately broken by irradiation prior to fertilization have given negative results (Glass, 1940). Cases of mosaic individuals have been described (Helfer, 1940) which seem to require delay of reunion until one or even two divisions after fertilization.

Several cases have been reported (Demerec, Kaufmann and Sutton, 1939; Demerec, Kaufmann, Sutton and Hinton, 1940; Demerec and Sutton, 1940; Kaufmann, 1939; Kaufmann and Bate, 1938) of rearrangements which have involved reunions of breaks in sister chromatids of irradiated chromosomes. In one case (Demerec, Kaufmann and Sutton, 1939) an individual was found to be mosaic for a reversed repeat in some nuclei and a corresponding deficiency in other nuclei. The possibility therefore exists that a considerable proportion of the exchanges occur between chromatid breaks and that cases of mosaic individuals owe their origin to this circumstance.

The yield of minute rearrangements, having breaks not more than 1% of the chromosome length apart, is proportional to the first power of the dose (Belgovsky, 1939; Belgovsky and Muller, 1937; Muller, 1940). Neutrons according to some experiments (Demerec, Kaufmann and Sutton, 1942) seem less effective than X-rays, but according to others (Dempster, 1941) they are slightly more effective. X-rays and γ-rays are equally effective (Muller, 1940). Ultraviolet light produces no exchanges in Drosophila (Demerec, Hollaender, Houlahan and Bishop, 1942; Mackenzie and Muller, 1940; Muller and Mackenzie, 1939).

Reports as to whether the temperature at the time of irradiation affects the yield of exchanges are conflicting, some experiments reported by Muller (1940) showing a reduced yield at a higher temperature while others show no difference, *e.g.* the experiments made by Makhijani. Other experiments (Mickey, 1938, 1939) show a definite increase in the frequency of exchanges induced by irradiation at a low temperature (4°C.) as compared with irradiation at a normal temperature. Alteration of the temperature at the time of fertilization has no effect on the yield of exchanges (Muller, 1940).

Post-treatment of irradiated sperm by exposure to near infra red radiation (Kaufmann, Hollaender and Gay, 1946) does not itself break the chromosomes or alter their capacity for reunion (by exchange or restitution) at the places of previous breakage, by X-rays. The post-treatment accelerates the attainment of maturity by the cells immature at the time of X-radiation. Since radiation induced exchange is less frequent in immature cells than in mature sperm, the net effect of the post-treatment is a reduction of the amount of exchange observed. When sperms are pretreated with near infra red for 24 or 72 hours and then exposed to X-rays an increased amount of exchange is observed. This augmented frequency might be an effect of the infra red in advancing immature and relatively less sensitive cells to a stage at which they are more sensitive. However the yields from sperm transferred at any stage over the 12–14 day interval required for sperm to become mature show no differences and certainly

no systematic decline, indicating that the sperm available for transfer the first few days after irradiation are a homogeneous group in respect to X-ray sensitivity. On the whole it appears likely that the infra red in some way sensitizes the chromosomes to X-ray breakage, though probably not through any heating effect.

Post-treatment of irradiated sperm with ultraviolet (2539 A) reduces the yield of exchanges (Kaufmann and Hollaender, 1946). Ultraviolet alone significantly raises the proportion of dominant lethals, and after X-rays produces the small rise of the order expected if the X-ray and ultraviolet dominant lethal production were cumulative. It is therefore inferred that the ultraviolet does not change the probability of reunion by promoting the survival of single breaks which act as dominant lethals (see Section XVII), but instead promotes restitution at the expense of exchange. Likewise, Swanson's (1944) data on Tradescantia suggest ultraviolet radiation increases restitution amongst breaks previously induced by X-rays, but does not affect isochromatid breaks.

XI. Production of Mutations by Radiations

Mutations have been produced by ionizing radiations in a great many organisms, the method of detection varying with the organism. With plant viruses, for example tobacco mosaic virus (Gowen, 1941), the leaves of a test plant have been inoculated with the irradiated virus solution. The test plant used was one which failed to give local necrotic lesions at the point of entry of the unchanged virus into the leaf. If some of the virus had been changed by the radiation into a form which did give local necrotic lesions, these would be detectable in the presence of the excess of unchanged virus. The lesions could then be excised for isolation and the permanence of the altered virus tested.

In the case of bacteria, the experiments have consisted essentially in plating out suitable dilutions of the irradiated bacteria on a solid nutrient medium, and examining suitable numbers of colonies. Each colony having grown from a single organism, it is presumed that a mutation in an irradiated bacterium would lead to a colony in which every individual had the mutant character. Mutations which lead to alterations in the size, colour or appearance of the colonies are recognisable by inspection, as in studies (Gowen, 1941) of changes in colony type in *Phytomonas stewartii*. Losses of biosynthetic capacity are detected by the isolates failing to grow on media containing only the bare minimum of substances required by the normal wild strain for growth (Tatum, 1945) in *Escherichia coli*, the specific mutation then being determined by the addition of various substances to the standard basic medium. Mutations to phage-resistance in *Escherichia coli* are detected (Demerec, 1946) by plating suitable dilu-

tions, phaging the dilution plates and determining the proportion of cells that survive to give colonies.

Amongst fungi, mutations have been induced by radiations in a variety of species, some of the more notable work being that in Neurospora where morphological and colour (Dobzhansky, 1936; Sansome *et al.*, 1945) and biochemical (Beadle and Tatum, 1945) mutations have been detected.

In diploid organisms the detection of recessive mutations, which constitute the majority, depends upon securing them in homozygous condition. This is not difficult in hermaphrodite organisms, such as many flowering plants, which may be self-fertilized. The detection of a recessive then depends only upon growing a suitably sized F_2 generation. By this means, radiation induced mutations have been detected in many plants' *e.g.* Antirrhinum (E. Bauer, 1932; Noethling and Stubbe, 1934, 1936; Stubbe, 1930), Nicotiana (Goodspeed, 1930), Hordeum (Stadler, 1928a; Muntzing, 1942), Zea (Stadler, 1928b). In flowering plants mutations affecting the gametophytes may often be detected in heterozygous condition (Buchholz and Blakeslee, 1930; Stadler, 1941) or *en masse* by increased variability of pollen grain size (Rick, 1942, 1943; Koller, 1943).

In the case of dioecious or self-sterile organisms more elaborate means must be adopted to secure recessives in homozygous condition, as for example in mice (Snell, 1935, 1939, 1941). The methods are relatively easy to use in genetically well-known organisms, such as Drosophila, mainly because a large number of special stocks are available.

Much the most extensive qualitative and quantitative information is available for *Drosophila melanogaster*. The usual procedure is to irradiate flies of one sex, normally the male, and to mate them to untreated members of the opposite sex. When this is done the cells of each zygote will contain one set of irradiated chromosomes and one set of control chromosomes. It is found that a proportion, dependent on the dose of radiation, of the eggs laid fail to hatch, that is in each egg that proved inviable a "dominant lethal" had been induced in the irradiated gamete nucleus (Catcheside and Lea, 1945; Demerec and Fano, 1944; Whiting, 1938). The nature of these dominant lethals is discussed in Section XVII.

Amongst the eggs which hatch and give viable adults, other genetic changes may be found. For example, a small proportion may appear morphologically different from normal flies through the possession of a dominant visible and viable mutation. These dominant changes are so called because they are manifested when only one of the two chromosome sets in an individual carries the change. A typical dominant visible change in Drosophila is the appearance in females of Notch wings. When such a female is crossed with a normal male, it is usually found that all her sons are normal and half her daughters Notch wing, the ratio of the sexes

being two females to one male, instead of the usual equality. The Notch gene is borne by the X-chromosome, of which the female has two and the male one. Besides the dominant visible effect, the mutant gene has a recessive lethal effect, for all efforts to secure males (hemizygous for it) and females with it in both X-chromosomes (homozygous for it) fail. Also most lethals are also cell-lethal, that is kill cells in which they are hemi- or homozygous in an otherwise heterozygous female fly.

Amongst the viable offspring of irradiated parents, there are also a considerable number of recessive lethal and visible mutations which produce no appreciable effect in individuals heterozygous for them, that is when borne by only one of the two sets of chromosomes. As the recessives are much more frequent than the dominant visible changes, they are more useful for quantitative experiments. However, special means have to be adopted in order to detect them.

For mutations at a particular locus, irradiated wild-type males may be mated to females homozygous for a recessive allelomorph of this locus. The vast majority of the offspring would be phenotypically wild type, but an occasional fly would show the mutant phenotype. This could be due either to a mutation of the wild type to a recessive allelomorph, or to the loss of the wild type locus through the induction of a deficiency. Suitable tests would differentiate between these alternatives. In fact deficiencies constitute a considerable proportion, about 30%, of "mutations" detected by this technique.

Visible, viable mutations in the X-chromosome can be studied very conveniently by mating irradiated wild-type males to attached-X females and examining the F_1 flies for mutant males. A sex-linked visible mutation induced in the X-chromosome of an irradiated sperm would be revealed by the male offspring which developed from a Y-bearing egg fertilized by that sperm. The proportion of mutations is underestimated by the attached-X method, partly because it is easy to overlook a single mutant fly in a culture especially where the mutant type differs only slightly from the wild type, but also because most mutants have a reduced viability.

Muller's ClB technique is more accurate. In females of the ClB stock, one of the X-chromosomes has an inversion, which greatly reduces recombination by crossing-over, a recessive lethal and the dominant marker Bar eye inseparable from the inversion. The recessive lethal ensures that a male zygote receiving a ClB chromosome will not survive. Irradiated males are crossed to ClB females, the Bar-eyed females of the F_1 selected and individually mated to any convenient male. Each of these females has two X-chromosomes, one ClB and the other an irradiated X-chromosome. The male offspring of each female are examined. None of the F_2 males can have the ClB chromosome, and all must have descendants

of the irradiated X-chromosome of the grandfather. If a mutation has occurred, all the males of the particular culture will show it. A recessive lethal mutation would be shown by the F_2 culture lacking males altogether; a semilethal by the proportion of males being significantly reduced below the expected one-third. Recessive lethals are induced about 10–20 times as frequently as visible mutations (Timoféeff-Ressovsky, 1937). Semilethals are induced at a rate approximately $2\frac{1}{2}$ times as great as that of the extreme lethals (Smirnov, 1934; Timoféeff-Ressovsky, 1934) but they are of little value for quantitative work owing to the difficulty of differentiating between slightly lowered viability and normality. The rates of X-ray induced lethals in *Drosophila funebris* (Timoféeff-Ressovsky, 1937), in *D. simulans* (Belgovsky, 1937; Demerec and Fano, 1944) and in *D. subobscura* (Christie, 1939) do not differ from those in *D. melanogaster*. In *D. subobscura*, the frequency of visibles is far higher than in *D. melanogaster*, the proportion of visibles to lethals in *subobscura* being about 0.43 (Christie, 1939).

We must consider the problem of establishing the statistical significance of inferences drawn from data about the induced rates of visible mutations where these rates are very small. Very often the device of attaching a standard error to an estimate is used to indicate the limits within which the true value may, at any chosen level of significance, be considered to lie. However the use of this in cases where the distribution is not normal, as with small mutation rates, may lead to erroneous conclusions. If a population contains a proportion, p, of a particular mutation then the probability of observing the mutation in any one random sample from the population is p. The probability of observing r mutants in a sample of size n is given by the binomial probability function

$$\frac{n!}{(n-r)!r!}p^r(1-p)^{n-r}.$$

The labour of computation involved in using this exact test to determine limits or to make tests of significance, is greatly reduced by the use of a table computed by Stevens (1942). Fryer and Gowen (1942) have given reasons why the use of the X^2 test in $2 \times n$ classifications is also satisfactory in many types of problem. In essence, the test will show whether deviations from expectation set up on various null hypotheses may reasonably be ascribed to random sampling variation. If the deviations are too great one may conclude that the hypothesis does not take account of the sources of non-random variation and further test may disclose what it is.

The rate of visible mutation induced at different loci is sensibly identical (Fryer and Gowen, 1942; Johnson and Winchester, 1934; Timoféeff-Ressovsky, 1932, 1933a and b, 1937; Timoféeff-Ressovsky and

Delbrück, 1936) except that a few loci seem more mutable than most others. Changes occurring at a rate greater than the average in *Drosophila melanogaster* are: $ct^+ \rightarrow ct$ (Fryer and Gowen, 1942) and $f \rightarrow f^+$ (Johnson and Winchester, 1934; Timoféeff-Ressovsky and Delbrück, 1936) in the X-chromosome, and possibly $dp^+ \rightarrow dp$ and $px^+ \rightarrow px$ in chromosome II (Fryer and Gowen, 1942). In so far as the genes studied are representative

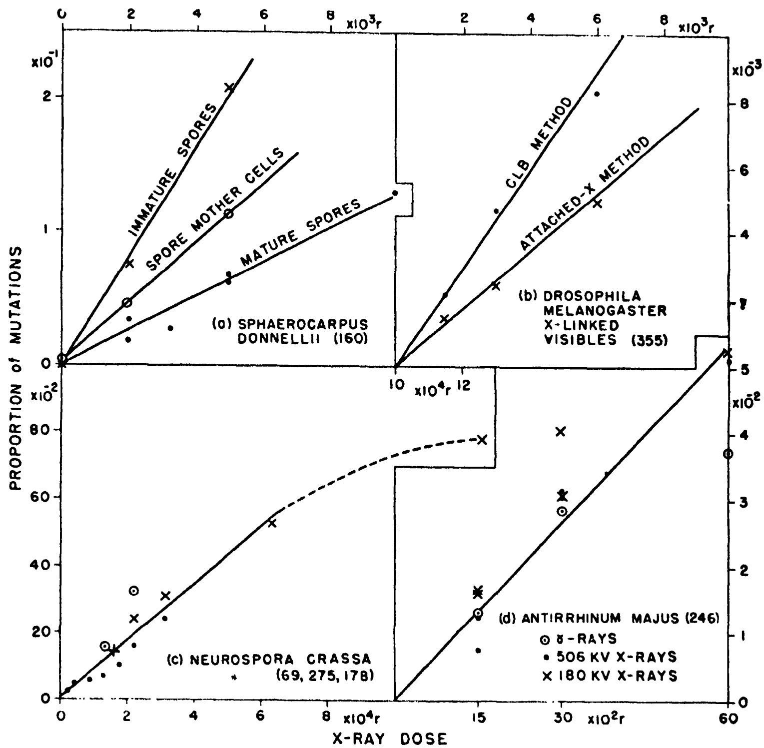


FIG. 17

Relation of the rate of visible mutation to dosage of X-rays.

of their respective chromosomes, the rates of mutation of genes on different chromosomes are the same. Data (Timoféeff-Ressovsky, 1933a and b) on the mutation rates of different allelomorphs at the w^+ locus in *D. melanogaster* show that the different allelomorphs mutate to other allelomorphs at the same rate. This rate is the same for mutations to all allelomorphs except to *w*, which has a probability of occurrence about three times that of other allelomorphs at the same locus. Schultz (1936) has suggested that the rate of mutation to and from a given allelomorph shows an inverse relationship approximating to an hyperbolic form,

Within the error of the experiments the proportion of visible mutations, induced by X-rays in a given organism, is linearly proportional to the dose (Drosophila, Timoféeff-Ressovsky and Delbrück, 1936; Hordeum, Stadler, 1928a, 1930; Antirrhinum, Stubbe, 1932, 1933; fig. 17). There is reason to believe that the yield of visible mutations produced by a given dose of X-rays is independent of the wavelength over the range 0.01–2.2 A (Fryer and Gowen, 1942). It is also probably independent of the intensity of the radiation and of whether it is fractionated (Patterson, 1931). These facts have been satisfactorily established in Drosophila for

TABLE 3

Mutation Constants (α)

Organism	Mutation	$\times 10^{-8}$	Reference
Tobacco mosaic virus	$+ \rightarrow$ aucuba	1.2	Gowen, 1941
	aucuba $\rightarrow +$	0.5	
Bacteria *Phytomonas Stewartii*	Average of 3 mutants affecting colony type	3.7	Gowen, 1941
B. coli	Phage resistance	0.1	Demerec, 1946
Drosophila melanogaster	$+ \rightarrow$ mutant	0.85	Johnson and Winchester, 1934
	$+ \rightarrow$ mutant	1.4	Timoféeff-Ressovsky, 1937
	$+ \rightarrow$ mutant	7.0–17.0	Fryer and Gowen, 1942
	$+ \rightarrow m$	2.4	Timoféeff-Ressovsky and Delbrück, 1936
	$m \rightarrow +$	1.0	
	$+ \rightarrow f$	6.6	
	$f \rightarrow +$	2.4	
	$+ \rightarrow w^e$	2.6	Timoféeff-Ressovsky and Delbrück, 1936
	$w \rightarrow w^e$	0.3	
	$w^e \rightarrow +$	0.8	
	$+ \rightarrow w^x$	15.9	Timoféeff-Ressovsky, 1933a and b
	$w \rightarrow w^x$	1.2	

sex-linked recessive lethal mutations. But since visibles are 10 to 15 times less frequent than lethals, adequate experiments to test the validity of extending the conclusions to the visibles would require an almost prohibitive amount of labour. So far as the experiments with visibles go, they show no sign of being in conflict with the conclusions based on recessive lethals.

In view of the proportionality of mutation rate to dose, the simplest way of expressing mutation rate is by stating a mutation constant defined (Timoféeff-Ressovsky and Delbrück, 1936) as the probability that a gene should mutate for a dose of one roentgen. The general agreement (Table 3) in order of magnitude between the mutation constants of organisms so different as Drosophila, bacterium and virus is striking. The same agreement is shown if total visible mutation rates in various organisms are compared (Table 4).

XII. Recessive Lethal Mutations in Drosophila

Determinations of the rate of induction of sex-linked recessive lethals by radiations under varying conditions have been made for a number of years by a considerable number of workers. The rate of induction of sex-linked lethal mutations is probably independent of the genetic stock used (Timoféeff-Ressovsky, 1940) and it is not correlated with the spontaneous rate. Where differences have been reported, there is sometimes a negative correlation with the rate of dominant lethal induction. For example, Oregon-R has a lower induced recessive lethal rate (Demerec and Kaufmann, 1937) and a higher dominant lethal rate (Demerec, Kaufmann and Hoover, 1938) than Swedish-b. The rate of induction of sex-linked lethals differs quite markedly from ripe to unripe sperm, being, for a dose of 3000 r, about 8.3% in ripe sperm and 1.5% in unripe sperm (ratio 1 to 0.181). With second chromosome lethals the difference is less marked, 20.9% in ripe and 12.2% in unripe sperm (ratio 1 to 0.584). These figures are means of determinations by several authors (Hanson and Winkelman, 1929; Lobasov, 1938; Neuhaus, 1934; Serebrovskaya and Shapiro, 1935; Sidorov, 1931). The differences may be the consequence of selection acting upon developing spermatogonial cells, as is suggested by the smaller difference observed where second chromosome lethals are concerned and the still smaller difference where sex-linked recessive visibles are studied (ratio 1 to 0.674). In any case, the differences observed emphasize the need for controlling closely the material used in making quantitative comparisons.

The proportion of lethals increases linearly with dose (Demerec, 1933; Efroimson, 1931; Oliver, 1932; Timoféeff-Ressovsky, 1931, 1934a; Zimmer, 1934; fig. 18). The most extensive block of data collected by one worker is that of Timoféeff-Ressovsky (1939b) based on approximately 60,000

TABLE 4

Mutation Rate per Nucleus per 1000 r X-rays

Organism	Tissue irradiated	Mutations detected	Mutation rate	Reference
			per cent	
Drosophila melanogaster	Sperm	Sex-linked visibles	0.15	Timoféeff-Ressovsky and Delbrück, 1936
		Sex-linked lethals	2.89	Timoféeff-Ressovsky, 1939b
		II chromosome lethals	7.3	Timoféeff-Ressovsky, 1937
		Total visibles	0.95 (estimated)	
		Total lethals	18.0 (estimated)	
		Total slight and semilethals	45.0 (estimated)	
Mouse	Sperm	Total visibles	2.0	Hertwig, 1939
Neurospora crassa	Microconidia	Total visibles	0.9	Demerec, 1946; Sansome, *et al*, 1945; Lindegren and Lindegren, 1941
Sphaerocarpus Donnellii	Mature spore tetrads	Total visibles	1.3	Knapp, 1935
	Spore mother cells	Total visibles	2.25	Knapp, 1935
	Young spore tetrads	Total visibles	4.1	Knapp, 1935
Field bean	Seeds (dry)	Total visibles	1.4	Genter and Brown, 1941
Antirrhinum majus	Pollen grains	Total visibles	0.9	Noethling and Stubbe, 1936

F_2 cultures. A straight line satisfactorily fits his experimental points, a χ^2 test of goodness of fit giving $P = 0.16$. However the experimental method detects chromosomes which carry lethals and does not differentiate between chromosomes with different numbers of lethals, therefore the curve connecting dose and the proportion of lethal chromosomes should not be strictly linear. The yield of lethal chromosomes should be proportional to $1 - e^{-mD}$, where m is the initial gradient (2.89% per 1000 r in this data) of the curve and D is the dose. A χ^2 test for the fit of such a curve to the experimental points give $P = 0.34$ (Lea, 1945).

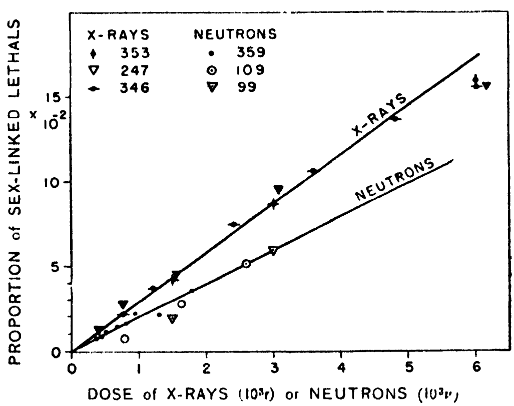


FIG 18

Relation of the rate of sex-linked recessive lethal mutations in Drosophila to dosage of X-rays or neutrons.

The effect of a given dose is independent of the intensity at which it is given over the range 0.009 to 2700 r per minute (Hanson and Heys, 1929a and b, 1932; Patterson, 1931; Ray-Chaudhuri, 1944; Timoféeff-Ressovsky, Zimmer and Delbrück, 1935; Timoféeff-Ressovsky, and Zimmer, 1935a; Wilhelmy, Timoféeff-Ressovsky and Zimmer, 1936), and of whether the dose is given continuously or is divided into a number of fractions separated by rest periods (Timoféeff-Ressovsky, Zimmer and Delbrück, 1935). In these experiments, the yield did not vary by more than a few per cent, if at all, for a 300,000 fold variation in intensity.

Experiments using X-rays excited at different kilovoltages down to 10 kv (namely awvelengths up to about 1 A), γ-rays and β-rays have not shown any difference in yield per roentgen of these radiations (Eberhardt, 1939; Fricke and Demerec, 1937; Hanson and Heys, 1929a, 1932; Hanson *et al*, 1931; Pickhan, 1935; Schechtmann, 1930; Timoféeff-Ressovsky and Zimmer, 1935b, 1938b; Timoféeff-Ressovsky, Zimmer and Delbrück, 1935). Experiments with still softer X-rays have shown a somewhat

smaller yield in some cases. The measurements of the dosage of these long wavelengths actually reaching the gonads are liable to error, especially on account of the uncertainty attaching to the estimate of the correction needed for absorption in the overlying tissues. Gowen and Gay (1933) obtained a yield of 1.2% per 1000 r with wavelengths 1.5 A and 2.3 A; compared with other data this value is probably too small. Wilhelmy, Timoféeff-Ressovsky and Zimmer (1936) obtained a yield of 2.23±0.3% per 1000 r using 2–3 A X-rays, but in view of the attendant difficulties in accurate dosage measurements the authors are doubtful whether the yield was really lower than the 2.89% obtained with harder X-rays. Fricke and Demerec (1937) found no difference between the rates induced by 0.94 A and 2.2 A X-rays.

Experiments with fast neutrons indicate that the latter are less effective than X-rays, for equal ionization in the tissue. However, the reliability of the experiments is not as good as that of X-rays, since neutron dosimetry is commonly less accurate. To make the results comparable, the neutron dosages must be expressed in a unit comparable to the unit (r) of X-ray dosage, *e.g.* in terms of Gray's (1943) "ν-unit," which is defined as that dose of neutrons that produces in water an ionization equal to that produced by 1 r of X- or γ-rays. The American "n-unit" is equivalent approximately to 2.5 ν-units, but the conversion factor varies slightly with different dosimeters.

The most extensive quantitative measurements are those of Timoféeff-Ressovsky and Zimmer (1938a and b; 1939b; Zimmer and Timoféeff-Ressovsky, 1938, 1942), which show that the yield for equal ionization in the tissue is only about two-thirds as large for neutrons as for X-rays. Data of other workers (Dempster, 1941; Giles, 1943; Fano, 1943b; Demerec, Kaufmann, Sutton, 1942) are in substantial agreement with this conclusion (fig. 18).

The suggestion has been made that, in addition to the quantitative difference in yield of mutations for equal ionization in the tissue, neutrons also differ from X-rays in that they show a tendency for a grouping of mutations to occur (Nagai and Locher, 1935; Nishina and Moriwaki, 1939, 1941). However the experiments indicate, contrary to the belief of their authors, that the distribution of lethal bearing sperms in different irradiated males is random and follows a Poisson distribution (Lea, 1945). Similarly there is probably no excessive tendency for more than one lethal to occur in a given X-chromosome, though one report gives contrary indications. Nishina and Moriwaki (1941) irradiated male flies with neutron doses giving lethals in 5 to 15% of the sperm and analysed some of the lethal chromosomes. They found that 4 out of 16 chromosomes bore more than one lethal, a proportion five times higher than is to be expected on

the basis of a random distribution of lethals in irradiated chromosomes. The probability of agreement with a hypothesis of no grouping is only 0.01, so that the hypothesis is contradicted. Fano (1943b), on the contrary, found no such grouping effect. Amongst 998 X-chromosomes analysed, 60 bore lethals, but only two carried more than one lethal. This proportion is in close agreement with the number expected if the lethals are randomly distributed amongst the chromosomes. Also the number of lethals recovered from each male irradiated showed no excessive grouping, the number of males with two or more lethal-bearing sperms not being significantly in excess of expectation.

The expectation of a grouping effect has its theoretical basis in the fact that when a tissue is irradiated by neutrons, the energy is dissipated by a comparatively small number of protons, each of which produces a large number of ionizations per micron of its path. Thus, it is assumed that the passage of a proton through the testes of a fly will be a rare event and that when it happens usually several sperm will be affected. Similarly that when a sperm is traversed often several mutations will be induced. However, the protons are much less rare than this hypothesis supposes. Thus, with 1000 ν-units of neutrons, about 4 protons traverse each square micron of tissue; so about 11 protons would pass through a sperm head of dimensions (Gowen and Gay, 1933) $7.4 \times 0.37\ \mu$. This dose induces X-linked lethals in 1.9% of sperm, so that the probability of a sex-linked lethal being produced in a sperm by a proton which crosses it is about 0.0017. The conclusion is that there is no experimental or theoretical justification for the belief that any grouping of the sorts looked for occurs with neutrons.

On the other hand there is quite a high probability that a proton, or indeed an electron, which produces a mutation, and which therefore presumably has traversed the chromosome, would pass through two or more adjacent turns of the spiral thread. The possibility therefore exists that lethals may occur together in compact groups, but such groups would be indistinguishable from single lethals by the methods used in the above experiments. The existence of such a grouping effect is rendered more likely by the fact that minute rearrangements are very frequently produced by radiations. Moreover, Muller (1940) has cited evidence of a grouping of induced mutations.

Experiments with α-rays (Beleites, 1939; Hanson and Heys, 1933c; Ward, 1935; Zimmer and Timoféeff-Ressovsky, 1936), which are even more densely ionizing than neutrons, are technically difficult on account of the short range (under 70 μ) of the rays in tissue. It was demonstrated (Zimmer and Timoféeff-Ressovsky, 1936) that mutations are produced when flies are kept in an atmosphere containing radon, but evaluation of

the dose received by the gonads was not possible. The increase in mutation rate is, however, proportionate to the time of exposure of flies to a radon atmosphere (Beleites, 1939). Measured irradiations of eggs have been made (Ward, 1935) at the polar cap stage when cells of the germ line in males are few in number (about thirty). A lethal induced in one of these cells would, following cell multiplication, be reproduced in a number of the mature sperms. Of 149 irradiated male flies tested, 7 carried lethals, the proportion of sperm with lethals varying in different flies between 8 and 100%. Reanalysis (Lea, 1945, and unpublished) of this data showed that the radiation produced 7 lethals in approximately 1000 X-chromosomes and that the yield of sex-linked recessive mutations induced by α-rays is $0.84 \pm 0.3\%$ per 1000 r. This is smaller than the yields obtained with X-rays (2.89% per 1000 r) or with neutrons (1.9% per 1000 r) and indicates that the yield of mutations, for equal ionization in tissue, decreases with increasing ion-density of the radiation. However, more strictly comparable experiments are needed since it is unsatisfactory to compare rates induced in sperm by X-rays and neutrons with those induced in polar cap cells by α-rays.

TABLE 5

Absence of Effect of Temperature on Rates of X-ray Induced Sex-linked Lethals in *Drosophila Melanogaster* (Timoféeff-Ressovsky and Zimmer, 1939a)

Dose (*r*)	*Temperature* (°*C*.)	*Number of X-chromosomes tested*	*Number of lethals*	*Per cent X-chromosomes with lethals*
1200	7	976	25	2.56
	32	921	29	3.15
2750	3	1821	154	8.46
	33	1645	134	8.15
3000	10	802	76	9.48
	35	736	60	8.15

The above facts summarize the principal tests by which one may recognize that the action of radiation in producing a mutation depends on a single ionization. The significant points are that the yield of mutations is strictly proportional to the dose, that for a given dose the yield is independent of the time over which the irradiation is extended and that the yield for a given ionization in the tissue diminishes with increase of ion-density of the radiation.

The conclusion that the mutation is directly due to ionization in the

gene is supported by the fact that the yield of X-ray induced mutations is independent of the temperature (Timoféeff-Ressovsky and Zimmer, 1939a, Table 5). It should be noted, however, that some authors have reported a higher frequency of induced mutation at low temperatures (Medvedev, 1935b).

XIII. Other Mutagenic Agents

The mutagenic action of various other physical agents has been tested with negative results, namely supersonic radiations (Hersh, *et al*, 1930), electricity (Horlacher, 1930), high frequency alternating potentials (Kerkis, 1935), electrostatic radiation (Schmitt and Oliver, 1933), heat irradiation (Grossman and Smith, 1933), ultra short radio waves (Whiting, 1937). Visible light when eosin is present as a sensitizer is said to increase the mutation rate in Antirrhinum (Stubbe, 1940), and low voltage cathode rays are stated to induce mutations in Aspergillus (Whelden, 1940).

The aging of tissues increases the spontaneous mutation rate in plants (Cartledge and Blakeslee, 1934; Peto, 1933) and Drosophila (Harris, 1929; Timoféeff-Ressovsky, 1935, 1937). So also does a temperature shock (Plough and Ives, 1934) and a moderate rise in temperature (Timoféeff-Ressovsky, 1935; Muller, 1928) for the whole life cycle. There is about a 6.5 fold increase in mutation rate of sex-linked lethals for a 10°C. rise in temperature in Drosophila (Timoféeff-Ressovsky and Zimmer, 1941; Timoféeff-Ressovsky, *et al*, 1935; Pätau and Timoféeff-Ressovsky, 1943) applied over the whole life cycle. The temperature coefficient for more frequently mutating genes is considerably lower, *e.g.* back mutation of *bb* (bobbed) has a temperature coefficient variously estimated at 4.0 (Timoféeff-Ressovsky and Zimmer, 1941) and 3.6 (Pätau and Timoféeff-Ressovsky, 1943).

Many chemicals have been tested to determine whether they possess mutagenic properties, in the great majority of cases with negative results. Most of the substances tested are more or less toxic to living organisms and it has been supposed that in sub-lethal treatments the physiology of an organism may be so disturbed that its genes become more mutable. Some substances have been employed which are assumed to affect amino groups in proteins. In Drosophila, the feeding of copper sulphate (Magrzhikovskaya, 1938), lead nitrate (Ponomarev, 1937, 1938), iodine (Kondakova, 1935; Sacharov, 1936a and b), acetic acid and ammonia (Lobasov and Smirnov, 1938a and b), and many other substances have been tried. Some positive results have been reported. The treatments were drastic enough to kill a great majority of the organisms treated, but the mutagenic effects where found have not been striking. In those cases where a positive result was found and the experiment repeated by independent

workers, no confirmation of the effect was secured. There are clear exceptions from this conclusion namely allyl isothiocyanate and mustard gas (Auerbach and Robson, 1944, 1946), phenol (Hadorn and Niggli, 1946) antisera (Emerson, 1944), and several carcinogens (Demerec, 1947), for each of which the evidence of marked mutagenic effects is very strong.

In the case of plants, there is evidence, in Antirrhinum, that culture of the plants in solutions lacking nitrogen, phosphorus and sulphur (Döring and Stubbe, 1938; Stubbe and Döring, 1938) leads to an increase of the general mutation rate. For fungi, chemical induction of mutations has been reported for Aspergillus species by means of nitrous acid, ninhydrin, chloramine T and hydriodic acid (Steinberg and Thom, 1940), it being assumed that these substances effect mutation through the destruction of groups in proteins. In these and similar experiments with fungi, in which mutant sectors have been obtained in cultures grown on abnormal media, there is no clear evidence which would ascribe mutagenic properties to the test substance rather than that it acts merely to select spontaneous mutants to which the abnormal medium was more favourable than to the wild strain. Thus, in the case of mutant sectors appearing in *Helminthosporium sativum* grown on media containing zinc sulphate, it was observed (Millikan, 1940) that the mycelium of the sector was undistorted and apparently normal while that of the parent colony was thickened and distorted on the medium containing zinc. When portions were transferred to media lacking zinc, the parent grew faster than the mutants. In Neurospora, the adaptation of a leucineless mutant to independence of leucine involves selection of mutants that occur spontaneously (Ryan and Lederberg, 1946).

It has previously been pointed out that the temperature of an organism at the time of irradiation is without influence on the induced mutation rate, at least in the case of *Drosophila melanogaster*. Also, starvation (Hanson and Heys, 1933a) and anaesthesia (Hanson and Heys, 1933b; Pickhan *et al*, 1936) do not modify the induced rate. However, other alterations in the physical or physiological conditions of the organism, cell and presumably the gene do effect the mutability of the gene to a marked extent. Swelling of the tissues by the imbibition of water results in an increased frequency of genetic changes induced by irradiation (Gelin, 1941; Genter, 1941; Gustafsson, 1941; Kaplan, 1939; Millikan, 1940; Stadler, 1931). In Epilobium, genes of *E. hirsutum* show a higher induced mutation rate when in a foreign cytoplasm (*E. luteum*) than they do in their own cytoplasm (Stubbe, 1935).

It has been assumed that the deposition of heavy atoms in an organism would lead to an increase of the mutation rate induced by radiation through an increased absorption of X-rays by the deposited atoms. This

TABLE 6

Effect of Feeding Fe, Pb and As to *Drosophila melanogaster* upon the Induced Rate of Sex-linked Lethal Mutation (ClB Method) for Different X-ray Energies

Experiment	*Number of F_2 cultures*	*Number of mutants*	*Mutation rate per cent*	*Differences*	*Reference*
3000 r (180 kV)	805	80	9.94 ± 1.05		
3000 r (180 kV) + 10% iron sugar	2756	357	12.95 ± 0.64	3.01 ± 1.23	Buchmann and Zimmer, 1940
3000 r (50 kV)	2640	251	9.52 ± 0.57		
3000 r (50 kV) + 10% iron sugar	4539	614	13.50 ± 0.51	3.98 ± 0.77	Buchmann and Zimmer, 1940
4000 r (180 kV)	353	39	11.05 ± 1.67		
4000 r (180 kV) + 0.4% lead acetate	473	53	11.20 ± 1.45	0.15 ± 2.2	Buchmann and Zimmer, 1941
4000 r (100 kV)	571	66	11.56 ± 1.33		
4000 r (100 kV) + 0.4% lead acetate	809	114	14.09 ± 1.22	2.53 ± 1.80	Buchmann and Zimmer, 1941
3240 r (70 kV)	1640	148	9.02 ± 0.71		
3240 r (70 kV) + 0.4% lead acetate	1779	245	13.77 ± 0.81	4.75 ± 1.08	Buchmann and Zimmer, 1941
3000 r (100 kV)	627	55	8.77 ± 1.13		
3000 r (100 kV) + As_2O_3 feeding	1133	139	12.25 ± 0.97	(1–2) 3.49 ± 1.49	
3000 r (180 kV) + As_2O_3 feeding	1335	122	9.14 ± 0.79	(1–3) 0.37 ± 1.38	Schütze, 1943

expectation has been realised in Drosophila, in experiments where larvae have been fed for a number of generations on media containing ferrum oxydatum saccharatum liquidum (Buchmann and Hoth, 1937; Buchmann and Zimmer, 1940), uranyl acetate (Buchmann and Sydow, 1940) or lead acetate (Medvedev, 1933, 1935a; Buchmann and Zimmer, 1941). Significantly larger numbers of sex-linked lethals were induced by a given dose of X-rays in metal-fed males as compared with control males. The validity of the assumption that the effect is due to an increased absorption of the X-rays by deposits of heavy metals could be tested in a number of ways: (a) by direct measurement of the absorption in the tissue; (b) by quantitative chemical analysis of the tissue and subsequent calculation of the energy absorption; (c) by investigating the dependence of the increased mutation effect on the atomic number of the metals fed; (d) by investigating the dependence of the increase of effect on the wavelength of the radiation used.

Determinations of the amounts of iron and of uranium in flies from stocks fed with these metals for a number of generations showed only a slight increase in their content of these metals (Born and Zimmer, 1940). Analysis for iron of the ash of gonads alone showed an insignificant increase in iron-fed flies. Similar results were obtained in analyses for lead in flies fed with lead acetate. The increase in X-ray absorbing power conferred by the extra amounts of metal is of the order of only 1% and is much too slight to account for the increased mutation rate of 30–50% on the supposition of a greater ability of treated flies to absorb X-rays.

Further, flies fed with iron showed no differential response to irradiation with hard and soft X-rays (Buchmann and Zimmer, 1940). This supports the conclusion that the increased mutational susceptibility due to iron-feeding cannot be explained by a mere increase in physical absorption. By contrast, the same authors (1941) have found that flies fed with lead acetate showed an increased rate of lethal mutation induced by subsequent X-ray treatment, the increase varying with the wavelength in accordance with the mass absorption coefficient of lead (Table 6). Similar relations are shown by arsenic-fed flies (Schütze, 1943).

It has been reported (Dotterweich, 1939, 1940) that flies fed with substances other than heavy metals and subsequently irradiated also show an increased induced mutation rate as compared with that in controls reared on normal food. Effective substances were pilocarpin (used as tincture of jaborandi leaves), caffein, sea-water and progynon. The chemical treatment alone gave no increased mutation rate. The X-ray treatment was given only once, after one or more generations had been reared in chemically altered food. The effect lasted and sometimes increased in magnitude during subsequent generations. For example (Dotterweich,

1940), when flies had been cultured for a number of generations with a 2% solution of tincture of jaborandi in the food and the males then irradiated with 2900 r, the sex-linked lethal mutation rate rose from the normal one of 8.5% to 13.6% after one generation of feeding and to 22.05% after five generations; thereafter it dropped, returning to the normal value in the twelfth generation. Similar, but less marked, effects occurred with weaker jaborandi solutions (0.5% and 1%) but with a more rapid return to the normal rate. The results were interpreted as showing a threshold effect of the alkaloid, which is made evident by X-rays, the return to normal being evidence of the occurrence of adaptive processes in the flies.

TABLE 7

Effect of Feeding *Drosophila melanogaster* for 5 Generations with Follicle Hormone (Progynon) upon the Frequency of Visible Mutations Induced by 4000 r X-rays (Dotterweich and Schmidtke, 1941a and b)

Concentration of progynon per c.c. food in ME (mouse units)	*Number of X-chromosomes tested*	*Number of mutants*	*Total mutants per cent*	*Number of each type of mutant*			
				Bristle	*Wing vein*	*Wing form*	*Body form and colour*
0	1374	15	1.12 ± 0.26	4	2	7	2
20	759	33	4.35 ± 0.74	17	13	0	3
80	1459	72	4.94 ± 0.68	58	3	9	2
2000	1684	133	7.99 ± 0.66	119	2	6	6

The visible mutations have been studied (Dotterweich and Schmidtke, 1941a and b) by the attached-X method in flies irradiated after cultivation for five generations on a medium incorporating various amounts of follicle-hormone, in the form of Schering-Kahlbaum's progynon. Few living progeny were obtained, but these showed an increase in the percentage of visible mutations over the progeny of control males irradiated and similarly mated. It was found (Table 7) that the increased percentage of mutants bore an exponential relation to the increase of dose of the progynon. When the mutations were classified into those affecting different characters of the body (see Table 7), it was clear that those affecting bristles were especially numerous. These bristle mutants consisted about equally of the sex-linked recessive scute and the autosomal dominant stubble, and demonstrate a highly specific effect of the follicle hormone.

On the contrary, Kanellis (1943) found no increased rate of radiation

induced sex-linked lethals in flies after they had been fed on the follicle hormone oestron, using a concentration of 2000 ME per cc. of food. Therefore, there exists not only uncertainty as to the mechanism by which abnormal feeding sensitizes the organism to radiation induced mutations, but also disagreement between authors as to whether the effect exists

XIV. Cosmic Rays and Mutations

After Muller (1927) had shown ionizing radiations could induce mutations, several workers (Muller, 1929; Gowen and Gay, 1933; Demerec, 1934a and b; Babcock and Collins, 1929; Hanson and Heys, 1930) supposed that the so-called spontaneous mutations might be caused by natural radiation, namely cosmic rays from outside the earth, γ-rays from the radioactive content of the environment, and α- and β-radiation from the radioactive matter in the organism itself. This possibility has been shown untenable in the case of Drosophila. The intensity of the natural radiation is insufficient (Muller, 1930a); generously estimated it is equivalent to about 2.5×10^{-8}r per sec., corresponding to about 0.05 r in the lifetime of a fly. With X-rays or γ-rays the rate of induction of sex-linked lethals is about 3% per 1000 r and is proportional to dose and independent of intensity over a very wide range. Thus 0.05 r could be supposed to produce 0.00015% of sex-linked lethals, which is about a thousandth of the observed natural rate in the less mutable races of *Drosophila melanogaster*. The calculation, based on an estimate of the intensity of the natural radiation derived from measurements of the natural leak of ionization chambers, takes no account of the fact that living organisms sometimes concentrate radioactive matter in their tissues. However, measurements of the radium content of flies (Muller, 1930a; Muller and Mott-Smith, 1930) show it to be insufficient to account for the spontaneous mutation rate.

Another argument arises from the fact that the rate of induction of mutations by radiation is independent of temperature, while the rate of spontaneous mutation is increased markedly by rise of temperature. It has also been shown directly (Rajevsky and Timoféeff-Ressovsky, 1939) that alteration of the intensity of radiation due to cosmic rays causes no detectable change in the rate of spontaneous mutation. Different groups of *Drosophila melanogaster* males were kept in wooden boxes (control), boxes with 15 mm. lead walls (the optimum for a cosmic ray shower effect) and boxes with 60 mm. lead walls (the pessimum for a shower effect) and then mated to ClB females. No significant differences were found. The results do not confirm the slight effects described by Jollos (1939), in whose experiments the material used was probably less closely comparable under the different conditions.

It therefore seems certain that spontaneous mutations in Drosophila

are not due to natural radiations. In some other less well investigated organisms there is some evidence pointing in the opposite direction (Rajevsky, *et al*, 1936).

XV. Radiations and Gene Size

When it was found that the production of mutations by X-rays was consistent with the view that a single ionization caused the mutation, the data were used to calculate a target size for mutation and it was supposed that the target should be identified with the gene. More recently, many writers have adopted a more cautious attitude and some have denied that the size of the target bears any relation to the size of the gene (Timoféeff-Ressovsky and Delbrück, 1936; Muller, 1940). When the target method is applied to the measurement of enzymes and viruses, objects which resemble genes and whose size may be determined by other independent methods, a very considerable measure of agreement, within a factor of 2 of the true diameter, is found between the sizes determined by the target and by the other methods (Wollman, *et al*, 1940; Lea, 1940b, 1945; Lea and Salaman, 1942; Lea and Smith, 1942). A method giving an estimate of gene diameter correct to a factor of 2 would be useful in view of the uncertainty attaching to the sizes determined by other methods. Those discussed by Muller (1935a), for instance, give only upper limits to the size of the gene.

There are two main reasons for doubting that the size of the target approximates the size of the gene (Lea and Catcheside, 1945b). Firstly it is supposed that ionization outside the gene may cause mutation, the inferred target size then being too big. For this effect to be of much importance, there must be enough spread of the effect of an ionization to give an appreciable chance of mutation in a gene due to an ionization occurring at least a gene diameter outside it. Secondly, it has been objected that ionization within a gene may produce mutation with considerably less than unit probability, so that the inferred target size would be too small (Sommermeyer, 1941).

It is known that the probability of inducing a mutation is proportional to the dose and independent of the intensity, and therefore that the mutation must be produced by the passage of a single ionizing particle. As the probability of mutation is independent of wavelength over a considerable range of the X-ray and γ-ray spectrum and γ-rays are at least as effective as more densely ionizing radiations, it is clear that a single ion cluster (Fano, 1942) is sufficient to cause a gene mutation. A majority of clusters are single ionizations, so that a single ionization, or at most two or three, are sufficient to cause mutation, though with what probability remains to be discovered.

The energy contributed to an atom through an ionization exceeds the energy necessary to break the chemical bonds holding the atom to its neighbours in the molecules; chemical change in the molecule may therefore be expected. In radiochemical studies, ionic yields of the order of unity are found to be typical (Lea, 1945). In some cases the ionic yield exceeds unity, through the initiation of a chain reaction so that more molecules react than there are ionizations produced. In other cases the ionic yield is less than unity, apparently through recombination of the products of dissociation of the molecule. However, it appears that with more complicated molecules exact restitution is less probable, so that in a large and complicated molecule, such as a gene is thought to be, ionization of an atom in it will almost inevitably produce a chemical change. It does not follow that such a change would always be detectable as a mutation.

Lethal mutation at a given locus appears to be not much more frequent than visible mutation (Patterson, 1932; Demerec, 1937; Muller, 1940). The recessive lethal is a change by which the gene or its characteristic activity is lost, that is, the gene is inactivated. The probability that an ionization produced in a gene leads to its inactivation is something that can be estimated roughly by appeal to experiments on inactivation of enzymes and viruses. If the two processes are analogous, the ratio of the size of the target to the size of the molecule would be equal to the probability that an ionization in the molecule will cause a lethal mutation. In determination of target and molecular weights of certain viruses, it was found (Lea and Salaman, 1942; Lea and Smith, 1942) that the two sizes agreed quite closely, the greatest difference being a factor of 5 in weight, which would be a factor of 1.7 in diameter if the body were a sphere. In each case the difference was in the direction of the target size being smaller than the true one, as would be expected if some of the induced changes left the virus still viable and infective. There is no evidence of the probability of mutation by an ionization in a virus being significantly less than unity.

In dealing with mutations at a specific locus only the probability of obtaining visible viable mutations is ordinarily possible. Being an underestimate of the total probability of mutation, the inferred gene size would be an underestimate also. Using the mutation frequencies of $1 - 10 \times 10^{-8}$ per roentgen (Table 3), the inferred molecular weight would be $10^4 - 10^5$ and the diameter if spherical 2 $m\mu$–6 $m\mu$.

In the case of sex-linked lethals at random loci, there exists the advantage of knowing the relative efficiencies of different radiations. There is, however, the disadvantage that some lethals (type B) are deficiencies some of which may be due to two neighbouring breaks, so that the lethals may have arisen without ionization in the gene itself. The pro-

portion of these cannot be estimated so this perturbation must be neglected.

If gene mutation can be induced by a single ionization, a radiation whose ionizations are widely spread would be more efficient per ionization than a more densely ionizing radiation which would be likely to give several ionizations within the same gene. This in fact is the relation that has been found experimentally. It is possible to derive an expression giving the target size (Lea, 1940a), using known values of the relative efficiencies of the radiations and of the spatial distribution of their ionizations. The estimate of target diameter so derived is extremely sensitive to errors in the determination of the relative efficiencies and may be affected by simplifications in the calculation. The most recent estimates (Lea, 1945) are given in Table 8; the order of target diameter 4–9 $m\mu$ is consistent with that inferred by the previous method.

TABLE 8

Relative Efficiency of Different Radiations in Inducing Sex-linked Lethals in *Drosophila melanogaster*

Radiation	*Per cent yield per 1000 r*	*Relative dose for equal yield*	*Inferred gene diameter ($m\mu$) (density = 1.35 g/cm^3)*	*Reference*
X-rays and γ-rays....	2.89	1.00	....	Timoféeff-Ressovsky, 1939b
Soft X-rays (2–3 Å)..	2.23	1.30	4.4	Wilhelmy, *et al*, 1936
Neutrons (Li + D) ...	1.90	1.45	9.0	Zimmer and Timoféeff-Ressovsky, 1938; Demerec, Kaufmann and Sutton, 1942; Dempster, 1941; Fano, 1943b; Giles, 1943
α-rays (ca 3 eMV)	0.84	3.44	6.6	Ward, 1935; Lea, 1940b

The number of genes in the X-chromosome, or more strictly the number of genes capable of yielding a recessive lethal mutation, may be derived from the X- or γ-ray dose required to give an average of one lethal per X-chromosome irradiated. This is $1000 \div 0.0289 = 3.46 \times 10^4$ r, since (Table 8) the yield of lethals per 1000 r is 2.89%. For a target diameter of 4 $m\mu$ the dose of X-rays (0.15 A) required to give an average of one mutation per gene is 2.9×10^7 r, and the number of genes in the X-chromosome would be $2.9 \times 10^7 \div 3.46 \times 10^4 = 838$. Larger target

sizes would mean smaller numbers of genes, for example for a target size of 6 $m\mu$ the number of genes would be 281.

These estimates are based on the assumption of unit probability for occurrence of a lethal mutation following ionization of a gene. Were the probability less than unity, say p, the estimate of the target diameter would be too small in the ratio of $p : 1$ and the estimate of the number of genes too great in the ratio $1 : p^2$ (Lea, 1945). There is little likelihood that the estimated number of genes is much too great, hence p^2 is not much less than unity and is very likely greater than 0.1. Hence p is most likely not less than 0.3 and most probably is nearly unity. Thus the gene diameter is most probably 4–8 $m\mu$ and it is unlikely that it exceeds 10 $m\mu$. Although the target theory is open to criticism, it may reasonably be said to have considerable heuristic value.

XVI. The Nature of Sex-Linked Recessive Lethals in Drosophila

It has generally been supposed that a recessive lethal mutation, like a recessive visible gene mutation, is an internal change in a gene which either gives an allelomorph which is lethal, or else destroys the capacity of the gene for reproducing itself so that it becomes lost, the loss being lethal when homozygous. The situation is, however, complicated by the fact that the recessive lethals do not form a homogeneous group, but include three types:

(a) The chromosome containing the lethal is structurally normal as far as can be determined by examination of the salivary chromosomes. This type may reasonably be interpreted as a mutation to a lethal allelomorph.

(b) The chromosome containing the lethal has a minute deficiency, revealed by the absence of one or more bands in the salivary chromosomes, the deficiency including the locus at which the lethal occurs (Slizynski, 1938). The smallest deficiencies, particularly those where a single band is deleted, may perhaps be caused by the destruction in a single gene of its capacity for reproduction. Somewhat larger deficiencies involving several bands are probably due to the simultaneous breakage of the chromosome at two places by the passage of a single ionizing particle. Still larger deficiencies, up to 50 bands in length and so reaching a fifteenth of the whole chromosome, are probably due to independent breakage of the chromosome in two places by separate ionizing particles. Deficiencies exceeding about 50 bands in length are not observed, probably because they behave as dominant lethals (Section XVII) and so are not viable even in the presence of a normal homologous chromosome.

(c) The chromosome containing a lethal is involved in a gross structural change (inversion or interchange), one of the breaks coinciding with the locus of the lethal or very close to it (Demerec, 1937; Demerec and Fano, 1941; Herskowitz, 1946; Oliver, 1932). Two alternative explanations of this case are (i) that the lethal is a position effect due to its separation from its normal neighbour or to its being brought into contact with a different gene and (ii) that the lethal is an internal change in the gene caused by the ionizing particle which broke the chromosome. The proportion of lethals associated with a structural change increases with increasing dose (fig. 19).

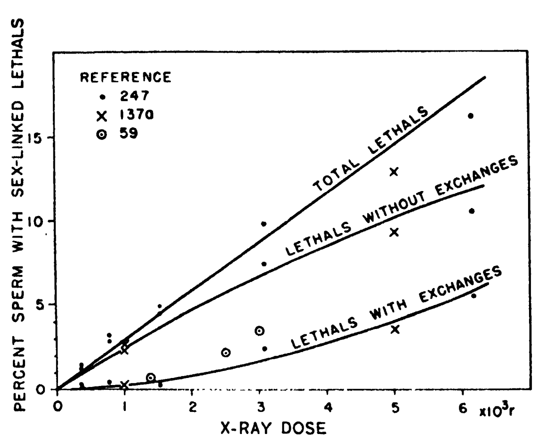


FIG. 19

Analysis of sex-linked recessive lethals in Drosophila into those associated with structural changes and those not so associated.

Lethals of class A, being produced by single ionizing particles, should increase linearly with dose. Those lethals of class B which are very small deficiencies induced by single ionizing particles, would also increase linearly with increasing dose (Belgovsky, 1939; Muller, 1940). A minority of class B lethals, having larger deficiencies caused by two separate breaks, induced by two ionizing particles, would increase in proportion to a higher power of dose.

If the association of a lethal with a gross structural change were due to a position effect, the lethals in class C would form a separate group the number of which would be proportional to the number of gross structural changes produced and cause no modification in the linear dependence on dose shown by class A and B lethals. This number increases more rapidly than the first power of the dose, since the structural changes depend upon two or more breaks which when due to X-rays are usually produced by

separate ionizing particles. Observation has shown (Section X) that in the dose range 1000–4000 r the number of gross structural changes is proportional to $(\text{dose})^{3/2}$. On the position effect interpretation, therefore, the observed lethals would be the sum of the two types, one unassociated with gross structural changes and with a frequency linearly proportional to dose, the other associated with gross structural change and having a frequency proportional to $(\text{dose})^{3/2}$. The total number would therefore increase more rapidly than the first power of the dose.

If however the lethals associated with gross structural changes were not fundamentally different from other recessive lethals, and the association with a structural change break depended only on the circumstance that the ionizing particle which caused the lethal also caused the break which took part in the rearrangement, the total number of lethals would be proportional to the dose. The number associated with gross structural change would increase in proportion to $(\text{dose})^{3/2}$; the residual lethals not associated with gross structural change would increase in proportion to less than the first power of the dose.

The data obtained by Timoféeff-Ressovsky (1939b), based on some 60,000 cultures, have been analysed (Lea and Catcheside, 1945a) from the above point of view. A χ^2 test of the experimental results was made to determine the maximum proportion of $(\text{dose})^{3/2}$ lethals that could be admitted. The results are shown in Table 9, in which the proportion of $(\text{dose})^{3/2}$ lethals postulated is indicated by stating in the first column the proportion of the total number of lethals which at 3000 r belong to this class.

TABLE 9

Analysis of Sex-linked Lethals into $(\text{Dose})^1$ and $(\text{Dose})^{3/2}$ Classes

Per cent of lethals at 3000 r which belong to $(\text{dose})^{3/2}$ class	χ^2	*Degrees of freedom*	*P*
0	2.3	2	0.32
12.5	3.6	2	0.16
17.5	6.4	2	0.04
22.0	9.6	2	0.008

The data provide no evidence for any $(\text{dose})^{3/2}$ term; a proportion as high as 17.5% is improbable and a proportion as high as 22% can practically be ruled out. Since some of the deficiencies of type B, which involve two independent breaks, account for some of the admissible $(\text{dose})^{3/2}$ component, it seems improbable that the type C lethals are position effects.

Experimentally the proportion of lethals associated with gross structural changes is found to be 0.35 ± 0.04 at 3000 r (Demerec, 1939; Demerec and Fano, 1941; Oliver, 1932). This proportion is far higher than the maximum proportion that can be reconciled with Table 9, so it may be concluded that the lethals associated with gross structural change do not constitute an additional class of lethals caused by position effect and requiring two ionizing particles for the expression of the lethal, but merely represent those cases where the ionizing particle which caused the lethal also caused a break which took part in structural change. Timoféeff-Ressovsky (1939b) has also considered the reasons for the lack of perturbation of the linear relation between dose and total yield of sex-linked lethals by those lethals associated with chromosome rearrangements.

It has been seen (Section VIII) that not all the chromosome breaks primarily produced take part in structural change, but that many of the broken ends rejoin and that the restituted chromosome is cytologically indistinguishable from an unbroken chromosome. Whether restitution or structural change occurs appears to depend only upon whether other breaks are available with which interchange can occur. It is therefore necessary to suppose that since some of the breaks which take part in structural change are lethals, so also some of the breaks which restitute are lethals. Such lethals would be recorded as type A lethals, *i.e.* without any cytologically detectable chromosome change. There is no reason to suppose that a type A lethal cannot be produced without the chromosome at the same time being broken. However, with the necessity on other grounds for a considerable number of restitutional breaks, a large part of the type A lethals must be restitutional breaks, so it would be worth testing whether *all* the type A lethals could be restitutional breaks.

Experimentally it is found that 3000 r produce in 1000 X-chromosomes 87 lethals, of which 39 are type A, 18 are type B and 30 are type C. The number of breaks in the euchromatin of the X-chromosome which take part in gross structural change when 3000 r is given to the sperm is 80 per 1000 X-chromosomes (95 according to Bauer, 1939). Of these, 30 are associated with lethals, namely the 30 type C lethals. We may infer that the probability that a chromosome break should cause a lethal is 30/80 = 0.38.

There are 18 minute deficiencies, the type B lethals, which are lethal presumably because one or more loci are deleted. It is reasonable to suppose that when a chromosome suffers two breaks, the segment between the break would be deleted or inverted with about equal probability (Demerec and Fano, 1941; Demerec, Kaufmann, Sutton and Fano, 1941). Thus corresponding to the 18 minute deficiencies, there would also be 18 minute inversions. An inversion would not be lethal *per se*, but since it involves

two breaks each of which has a probability 0.38 of being a lethal, the probability is $0.38 + 0.38 \times 0.62 = 0.63$ that at least one of the breaks will be associated with a lethal. Hence of the 18 minute inversions 11 ($= 18 \times 0.63$) would behave as lethals and would be included in the type A lethals, as a minute inversion is unlikely to be detected cytologically. Slizynski (1938) suspected some of the non-deficient lethals were minute inversions.

Consequently since 11 of the 39 type A lethals are minute inversions, the remaining 28 would be restituted breaks. But we have seen that only 38% of breaks produce lethals, so the total number of restituted breaks would be $28/0.38 = 74$. The total number of breaks of all sorts produced by 3000 r in the euchromatin of 1000 viable X chromosomes is therefore 226, consisting of 36 in minute deletions (2 breaks per deletion), 36 in minute inversions, 80 in gross structural changes and 74 which restitute. The proportion which take part in gross structural changes at 3000 r is $80/226 = 35\%$; this proportion would be greater at higher doses and smaller at lower doses.

It should be noted that there are 18 minute deletions to 74 restitutional breaks, a ratio of 1 to 4. In her cytological study of inversion breakage points Hoover (1938) found 5 breakage points with deletion to 15 without, a ratio of 1 to 3. The agreement of these ratios suggests that the number of restitutional breaks have not been greatly overestimated by assuming all A type lethals are restitutions.

Herskowitz (1946) has reached similar values, arguing that if the probabilities of a break (B) being lethal (L) or of entering into a rearrangement (R) are independent, the product of these frequencies would be the frequency with which a break produced a lethal and also entered into an observed rearrangement (LR), *i.e.* $\frac{L}{B} \cdot \frac{R}{B} = \frac{LR}{B}$. From available data on L, R and LR, the number of breaks involved may be calculated. For 3000 r, using the previously quoted data, there are 232 breaks per 1000 viable X chromosomes. The breakage rates inferred by Herskowitz show a linear proportionality to dosage between 1000 r and 6000 r.

Any break irrespective of its subsequent history, namely as to whether it restitutes, takes part in a minute deletion or inversion, or takes part in a gross structural change, may result in a lethal change in the gene at or adjacent to the breakage point, the change sometimes being destruction of the power of reproduction. The probability of a break causing a lethal in this way is 0.38. Consequently any type of structural change is liable to have a lethal change in the gene adjacent to a breakage point. In

addition to this effect, a deletion will usually have a recessive lethal effect on account of the absent loci.

XVII. Dominant Lethals

An egg fertilised by a sperm carrying a dominant lethal will not give rise to an adult organism and normally the dominant lethal causes the death of the zygote at a fairly early stage. In consequence a dominant lethal cannot be maintained and studied in successive generations. The occurrence of dominant lethals in Drosophila sperm, for example, can only be inferred from the observation that a proportion of the eggs fertilised by such sperm give inviable zygotes, shown by some of the eggs failing to hatch or to develop into adult flies.

A number of experiments (Catcheside and Lea, 1945a; Demerec, Kaufmann and Hoover, 1938; Fano and Demerec, 1941; Gowen and Gay, 1933; Hanson, 1928; Sonnenblick, 1940) have been made to determine how the proportion of eggs which hatch, or which reach pupal or adult stages, diminish with increasing dose of radiation received by the sperm which fertilised the eggs (fig. 20). The close agreement of the rates for

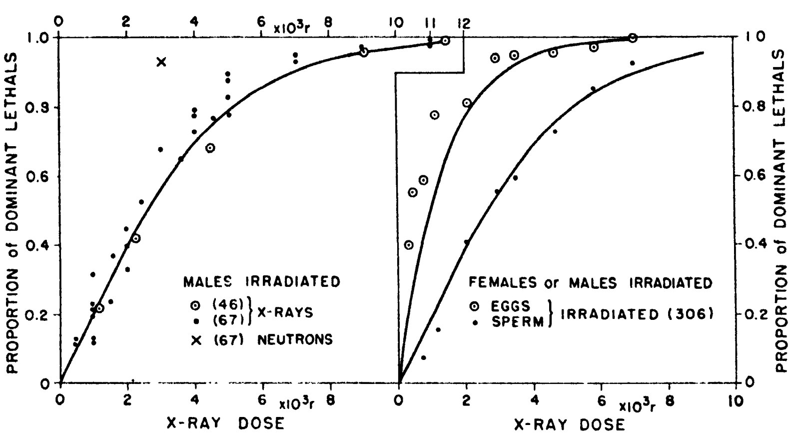


Fig. 20

Relation of the rate of dominant lethals to dosage of X-rays.
Points experimental, curves theoretical (Lea and Catcheside, 1945a and unpublished).

death in the egg and at later stages respectively shows that death caused by irradiation of the sperm usually occurs in the embryonic stage, those organisms that survive to the larval stage normally developing into adults. Sonnenblick (1940) has shown that the eggs which do not hatch show abnormal mitotic divisions with clumped and broken chromosomes and bridges.

Considerable differences exist between the frequencies of dominant lethals observed in replicate experiments, greater than is to be expected in the basis of sampling errors (Catcheside and Lea, 1945a; Demerec and Fano, 1944). The reasons for the quantitative uncertainty are not definitely known, though Dempster (1941) states that the sensitivity of the sperm to the induction of dominant lethals increases with the age of the male. Neutron irradiation apparently induces a greater proportion of dominant lethals than does X-ray irradiation at comparable doses (Fano, 1944).

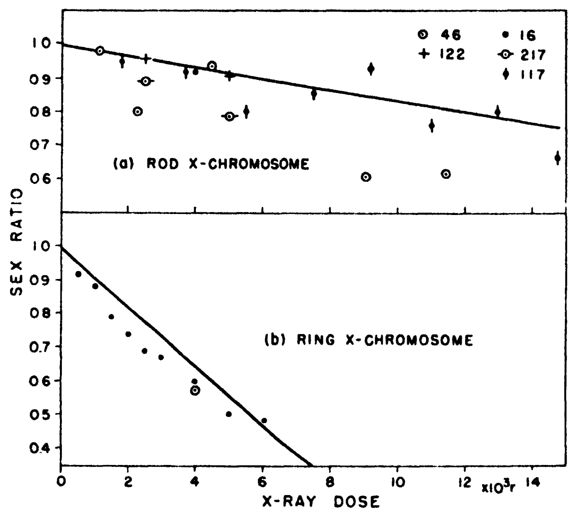


Fig. 21

Relation of the sex ratio depression in rod and ring X-chromosome stocks to the dosage of X-rays. Points experimental, curves theoretical (Lea and Catcheside, 1945a).

The evidence that the effect is genetic rather than physiological is as follows (Muller, 1940). The sperm is composed almost entirely of chromatin, the volume of the sperm head being about equal to the combined volume of the chromosomes at meiotic metaphase. The action of the radiation on the sperm can hardly be on cytoplasm or nuclear sap. Secondly, the proportion of female flies hatching from eggs fertilised by irradiated sperm is reduced more than the proportion of male flies (fig. 21) showing that the X-bearing sperm are more sensitive than the Y-bearing sperm. If the action is a genetic one on the chromosomes, this result is to be expected since fewer breaks are produced in the Y chromosome than in the X, and it is genetically almost inert. Further, the reduction in the sex ratio is particularly marked when the X-bearing sperm carries a ring X-chromosome instead of the normal rod (Bauer, 1939b and c; Catcheside

and Lea, 1945b). Finally, a consistent quantitative interpretation of the curve relating the yield of dominant lethals to the dose can be obtained on the basis that the cause of dominant lethals is the breakage of chromosomes by the radiation (Demerec, Kaufmann, Sutton and Fano, 1941).

Dominant lethals are chiefly, if not exclusively, chromosome structural changes, rather than gene changes. Small deficiencies usually have a recessive lethal rather than a dominant lethal effect, but at least one case of dominant lethality has been found (Alikhanian, 1937). It does not seem probable that a gene mutation would have a more strongly lethal effect than a complete deficiency for the gene concerned, so dominant lethal mutations in the strict sense of intra-gene changes are probably rare. Larger deficiencies, involving more than about 50 salivary bands, and dicentric chromosomes do not occur in viable zygotes. There is evidence to show (Demerec, Kaufmann, Sutton and Fano, 1941) that deficiencies are induced as frequently as inversions of similar size and asymmetrical interchanges as frequently as symmetrical ones. It must be concluded that the large deficiencies and asymmetrical interchanges (Pontecorvo and Muller, 1941) behave as dominant lethals. However, calculations (Fano, 1941) based on the frequencies found for the observable types of aberration show that these particular aberrations are not nearly frequent enough to account for the whole of the observed dominant lethals. Further, the shape of the survival curve is inconsistent with the assumption that dicentric interchanges and large deletions account for any considerable proportion of the dominant lethals. When the survival curves (Catcheside and Lea, 1945a and b) are plotted semilogarithmically, they are initially linear. This suggests that a single ionizing particle causes the effect. Since a large interstitial deletion or an interchange involves the production of two chromosome breaks usually by two separate ionizing particles, it must be concluded that there is a more important mechanism, involving a single ionizing particle, which also has a dominant lethal effect. At small and moderate doses where the curve is approximately linear, this effect predominates; at larger doses the two break aberrations, which increase in frequency more rapidly than the first power of the dose, become more important, thus accounting for the increased gradient shown at higher doses.

Chromosome loss, caused by a single chromosome break, has been proposed (Muller, 1940; Muller and Pontecorvo, 1942; Pontecorvo, 1941, 1942) as the mechanism concerned. A proportion of breaks neither restitute nor rejoin in a new way, but remain open, and when the chromosome divides, a proportion of the broken ones show sister union of the chromatid ends at the breakage points. At anaphase there is an acentric fragment and a dicentric chromosome, and such chromcsomes are likely

to be lost at cell division. The evidence indicates that it is not so much the loss of the chromosome that is lethal, as the process of loss.

In *Drosophila melanogaster*, the loss of a chromosome is certainly lethal in the case of the large autosomes II and III though a fly can exist in the absence of one of its small chromosome IVs. Also, XO males are viable and may be interpreted as normal males which have lost a Y-chromosome or as normal females which have lost an X-chromosome. However, about 80% of all the breaks induced in sperm occur in chromosomes II and III, so persistent single breaks would usually behave as dominant lethals.

Experiments (Muller, 1940; Pontecorvo, 1941) have shown that chromosome losses induced by single ionising particles do occur. The percentage of losses induced was found to be linearly proportional to the dose, showing that the loss occurred by a single break process. However, the number of viable losses is small, much smaller than the number of breaks expected in the sex chromosomes on the basis of the number of breaks in the autosomes required to explain dominant lethals. As shown by comparison with the amount of change in the sex ratio caused by equivalent doses, the lethal losses are probably three times as numerous as the non-lethal ones. It must be concluded that though the complete absence of one sex-chromosome from a zygote may be viable, the process of loss often causes death. The explanation is that the chromosome bridge formed when sister union has occurred usually causes a mechanical upset of mitotic division. It has been suggested (Demerec and Fano, 1944) that death occurs through formation of polyploid cells as a consequence of a persistent bridge leading to a single restitution nucleus instead of separate daughter nuclei.

The viable losses, to the extent of about 1% per chromosome at 4000 r (Pontecorvo, 1942), occur apparently only when the break occurs close to the centromere, so that a very short bridge would be formed at anaphase. The daughter nuclei could then be formed without interference from the chromosome in process of loss. Several kinds of evidence support this, for instance an X-chromosome deficient for all regions to the left of *B* (Bar) suffers viable loss as frequently as a whole X.

Loss of all chromosomes at high doses of irradiation, resulting in failure of some sperm to fertilize and thus kill the eggs, may lead to a decrease in "dominant lethals" with increasing dose (Maxwell, 1938; Whiting, 1945) in organisms (*e.g.* Habrobracon) where embryos may be haploviable. However, biparental offspring whether female or male regularly decrease in frequency with increasing dose of X-rays to the sperm (Whiting, 1938a).

A mathematical expression for the yield of dominant lethals as a function of the dose of radiation given to the sperm has been derived (Lea and Catcheside, 1945a) on the basis of the mechanisms of unjoined single breaks and asymmetrical exchanges. Simplifying assumptions were used to make the analysis manageable, namely that only one break may occur in each chromosome arm and that joining between the various broken ends occurs at random. The first is not a serious source of error as is shown, at the cost of heavily increasing the calculations, when the assumption is not made (Haldane and Lea, 1947). The second assumption may be reasonable for Drosophila sperm, but not for all materials.

Let p be the probability that a given break shall neither restitute nor interchange, but shall lead to chromosome loss and to a dominant lethal effect; $q(= 1 - p)$ is the probability it shall either restitute or interchange. If there are r breaks in a cell, q^r is the probability all either restitute or interchange.

If, with dose D, the mean number of breaks per sperm is $m = \alpha D$, the proportion of sperm having r breaks per sperm is given by the Poisson distribution and is $e^{-m} \cdot m^r/r!$ The probability that a sperm will have no break is e^{-m}. The probability that it will have one break is $m \cdot e^{-m}$. Sperm with one break will contribute $(1 - q)m \cdot e^{-m}$ to dominant lethals, and $qm \cdot e^{-m}$ to viable zygotes without aberrations.

A proportion $\frac{1}{2}m^2 \cdot e^{-m}$ sperm will have two breaks per sperm; $(1 - q^2)\frac{1}{2}m^2 \cdot e^{-m}$ of these will be dominant lethals owing to the failure of one or both breaks to restitute or exchange. Of the $q^2 \cdot \frac{1}{2}m^2 \cdot e^{-m}$ in which all four broken ends join at random according to our assumption, one-third will be contributed to each of the classes (a) restitution, giving viable sperm without aberration, (b) symmetrical interchange, giving viable sperm with aberration, and (c) asymmetrical interchange, giving inviable sperm and so adding to the dominant lethals. Thus the sperm in which two original breaks were induced yield with normal eggs $1/6\,m^2 \cdot q^2 \cdot e^{-m}$ normal viable zygotes, $1/6m^2 \cdot q^2 \cdot e^{-m}$ viable zygotes with chromosome aberrations, and $\frac{1}{2}m^2 \cdot e^{-m}(1 - \frac{2}{3}q^2)$ dominant lethal zygotes.

In general there would be $e^{-m} \cdot m^r/r^1$ sperm with r breaks. In $e^{-m} \cdot m^r \cdot q^r/r^1$ sperm no breaks will remain unjoined. In a sperm of this class the r breaks may join in $1 \cdot 3 \cdot 5 \cdot \cdot \cdot (2r - 1) = \dfrac{(2r)!}{r!\,2^r}$ ways, of which one way is viable without aberration, $(r! - 1)$ ways viable with aberration and the remainder inviable (Catcheside, 1938a; Fano, 1943a).

Summing the contributions from sperm with various numbers of breaks, and replacing m by αD we have:

Proportion of sperm which yield normal viable zygotes is $X = e^{-\alpha D} S_1$,

where $S_1 = 1 + \alpha qD + 1/6(\alpha qD)^2 + \cdots + \frac{(2\alpha qD)^r}{(2r)!} +$

Proportion of sperm which yield viable zygotes is $Y = e^{-\alpha D} S_2$,

where $S_2 = 1 + \alpha qD + 1/3(\alpha qD)^2 + \cdots + \frac{(2\alpha qD)^r \cdot r!}{(2r)!} + \cdots$

Total number of primary breaks formed in viable sperm, per total sperm is

$$Z = e^{-\alpha D} S_3,$$

where $S_3 = \alpha qD + 2/3(\alpha qD)^2 + \cdots + \frac{(2\alpha qD)^r \cdot r \cdot r!}{(2r)!} + \cdots$

The various series, S_1 to S_3, can be summed, the results being as follows:

$$S_1 = \cosh \sqrt{(2\alpha qD)}; \quad S_2 = 1 + \sqrt{(\tfrac{1}{2}\pi\alpha qD)}\, e^{\frac{1}{2}\alpha qD} \operatorname{erf} \sqrt{(\tfrac{1}{2}\alpha qD)};$$

$$S_3 = \tfrac{1}{2}\alpha qD \left\{ 1 + \frac{1 + \alpha qD}{(\frac{1}{2}\alpha qD)} \frac{\sqrt{\pi}}{2} e^{\frac{1}{2}\alpha qD} \operatorname{erf} \sqrt{(\tfrac{1}{2}\alpha qD)} \right\};$$

where

$\cosh x = \frac{1}{2}(e^x + e^{-x})$ is the hyperbolic cosine and $\operatorname{erf} x = \frac{2}{\sqrt{\pi}} \int^x e^{-x^2} dx$

is the error-function. Values of S_1, S_2, and S_3 have been tabulated for various values of αqD (Lea, 1945).

The proportion of viable sperm which have exchanges may be observed (Catcheside, 1938a; Bauer *et al*, 1938); the theoretical proportion expected is $(1 - X/Y) = (1 - S_1/S_2)$, and may be tabulated as a function of αqD. Fig. 16a shows the experimental data compared with the theoretical curve $(1 - S_1/S_2)$ fitted to the data by taking $\alpha q = 0.57$ per 1000 r.

The proportion of sperm which yield dominant lethal zygotes is $(1 - Y)$ and this curve together with experimental points (Catcheside and Lea, 1945a; Demerec and Fano, 1944) is plotted in fig. 20. In computing Y, the value of αq is already known to be 0.57, so S_2 can be calculated for each dose using the published table (Lea, 1945). Although αq is fixed, α is arbitrary; but the value $\alpha = 0.75$ was found to give the best fit of the theoretical curve to the experimental points. Hence $q = \frac{0.57}{0.75} = 0.76$. It has now been deduced that $\alpha = 0.75$ is the number of primary breaks produced per sperm per 1000 r; $q = 0.76$ is the probability that a break will join, either by restitution or by exchange; and $p = 1 - q = 0.24$ is the probability that a break will remain unjoined and behave as a dominant lethal.

The mean number of breaks primarily formed per viable sperm is S_3/S_2 and may be calculated using $\alpha q = 0.57$. The results are given in

Table 10. From these figures the mean number of breaks in the euchromatin of the X-chromosome may be obtained, using the result that a proportion 0.162 of all observed breaks occur there (Fano, 1941). At 3000 r, $0.162 \times 1.23 = 0.199$ primary breaks occur in the euchromatin of X-chromosomes. This is in reasonable agreement with the estimate of 0.226 per sperm derived on the hypothesis that recessive lethals are rejoined breaks.

TABLE 10

Dose (r)	1000	1500	2000	3000	4000	6000
Mean number of breaks per sperm	0.75	1.13	1.51	2.25	3.0	4.5
Mean number of primary breaks per viable sperm	0.49	0.69	0.89	1.23	1.54	2.15

It has been mentioned already that in the progeny of irradiated male flies there are fewer females than males, indicating the production of more dominant lethals in the X- than in the Y-chromosome, though the data of Demerec and Fano (1944) cast doubt on this conclusion. This sex difference may be ascribed to the fact that more breaks occur in the X- than in the Y-chromosome in the ratio 100 : 76 (Bauer *et al*, 1938). The calculations outlined above can be extended to this case (Lea and Catcheside, 1945a) and the theoretical curve, together with experimental values, is given in fig. 21.

If the irradiated males have a ring X (X^{c2} was used in the experiments) instead of a rod X, the sex-ratio distortion is much more extreme (Bauer, 1939b; Catcheside and Lea, 1945b). This is due to the fact that not only does a break which fails to join produce a dominant lethal, but so also does a proportion of the breaks which restitute. It is known from a comparison of the relative frequency of inversions in ring-X and rod-X chromosomes (Catcheside and Lea, 1945b) that about half of the restitutions are inviable. This appears to result from restitution occurring at, or subsequent to, division of the chromosome so that half the restitutions would form a double-sized dicentric chromosome when the centromere divides; this abnormal chromosome behaves as a dominant lethal. Therefore the probability that a single break will produce a dominant lethal is $p + \frac{1}{2}q$ in the case of a ring chromosome, compared with p in the case of a similar sized rod chromosome.

Using these facts, the sex-ratio depression in a ring-X stock can be calculated as previously, the theoretical curve and experimental values being shown in fig. 21 (Lea and Catcheside, 1945a). Further, from the

observed proportion of the sex-ratio depressions in rod-X and ring-X stocks, the ratio of $p + \frac{1}{2}q$ to p may be obtained and so values of $p = 0.26$ and $q = 0.74$ obtained (Catcheside and Lea, 1945b). These are in very good agreement with the values obtained by the previous method. By a further development of the argument it is possible to infer that the number of breaks primarily produced per sperm per 1000 r is 0.78.

Irradiation of eggs produces, for a given dose, more dominant lethals and fewer structural changes than does sperm. Now, it is to be expected that the constant α would be the same in sperm and egg, but it is possible that p and q may be different. It is reported (Glass, 1940a) that irradiation of the egg results in some inversions but no interchanges. Since the theory uses the simplification that only interchanges are counted, it may be assumed as a first approximation that $q = O$ and $p = 1$ in eggs. The expression $1 - e^{-\alpha D}.\ S_2$ for the yield of dominant lethals in sperms thus simplifies to $1 - e^{-\alpha D}$ for eggs (Lea and Catcheside, unpublished). The resultant curves (using $\alpha = 0.75$, $q = 0.76$ for sperm and $q = 0$ for eggs) fit Sonnenblick's data (1940), if it is assumed his dosages are 50% too small as is indicated by comparison with determinations of other workers.

XVIII. The Nature of Visible Mutations

Minute deficiencies often simulate mutations at the same locus (McClintock, 1938b, 1941c, 1944b; Muller and Raffel, 1935). Usually such deficiencies act as recessive zygotic lethals and gametophyte lethals in plants but if the deficiency is very small it may be viable in the female gametophyte and even in the male gametophyte. Several cases of these kinds have been described by McClintock (McClintock, 1942b, 1944a and b) for deficiencies, in the short arm of chromosome 9 in maize, which have arisen through breakage, sometimes repeatedly, in a bridge-break-fusion cycle followed ultimately by healing of the broken ends. Quite large deficiencies may be transmitted by the female gametophyte, though often with a lower frequency than non-deficient chromosomes. The rate of functioning seems to be remarkably sensitive to the environment. Pollen grains that are deficient for small terminal segments (McClintock, 1942b) of chromosome 9 are completely functional. Those with larger deficiencies, though entirely normal in appearance, do not function. In cases where the terminal deficiency functions in both gametophytes, homozygous viable embryos may be secured. The seedlings grow normally at first, but into pale yellow or white seedlings, according to whether the deficiency is smaller or larger. The seedling dies when the food reserve in the endosperm is exhausted. There is little doubt that the deficiencies simulate unknown amorphs (Muller, 1930b) of normal genes in the region. In another case (McClintock, 1944b) a moderately large interstitial deficiency, which is

somewhat viable in the male gametophyte mimics, when homozygous, a known recessive gene, *bz* (bronze). Mimics of the gene yg_2 have also appeared in the same experiments, some of them definitely associated in origin with the occurrence of breaks in the usual bridge-break-fusion cycle.

In maize it has also been possible to demonstrate the production by X-rays (Weaver, 1942) of deficiency mutants that resemble in all particulars some mutants which appear by loss of portions of minute centric ring chromosomes (McClintock, 1938b).

Some (Serebrovsky, 1929) have been led to suppose that "gene mutations" might be nothing more than minute deletions and other rearrangements, of fundamentally the same kind as the large visible ones. The evidence from reverse mutations in Drosophila (Timoféeff-Ressovsky, 1937), though not in maize (Stadler, 1944), raised objections to such a view, but crucial evidence is very difficult to obtain since reversibility might be possible in the case of a rearrangement, such as a minute inversion. Thus, not only are scute mutations sometimes deficiencies (Muller, 1935b) but also some induced ones are minute inversions (Muller, Prokofyeva and Raffel, 1935).

Some other evidence also suggests visible and lethal mutations constitute substantially different classes of events. Thus, amongst a series of 61 X-ray induced lethals at known loci, there were 26 with a gross chromosomal aberration in the same chromosome (Demerec, 1937). In all but two of these 26 cases the lethal was at one of the breaks of the aberration. By contrast, amongst a series of 30 visibles which appeared in the same experiments, only one had a chromosomal aberration, neither break of which was associated with the visible mutant. It has already been seen that a majority, if not all, of the X-ray induced recessive lethal mutations in Drosophila are associated in origin with the occurrence of breaks. The evidence suggests that the recessive visible changes are not so associated. This is supported by the finding (Demerec, 1939b) that viable changes in the white-notch region, besides being unassociated with gross chromosomal aberrations, are also free from the deficiencies which are so common amongst recessive lethals.

The "dominant" visible mutants, often lethal when homozygous, are nearly always structural changes of some kind. They may be minute deficiencies, *e.g.* many Notch mutants (Demerec and Fano, 1941); or small duplications, *e.g.* Bar, eyeless-Dominant and Hairy wing; or small inversions, *e.g.* Dichaete. Sometimes, *e.g.* Curly, they are associated with large inversions, from which the mutant is inseparable. It is probable that many such changes are position effects, though definitive evidence cannot be provided. Several position effects with a recessive effect have been proved most elegantly (Panshin, 1935; Dubinin and Sidorov, 1935) and

some scutes (Muller, 1935b; Muller, Prokofyeva and Raffel, 1935) may be similarly interpreted.

It has previously been pointed out that minute rearrangements (deficiencies and inversions) occur far more frequently than is to be expected on the basis that they are merely the extremely small variants of rearrangements randomly distributed as regards size. They are therefore a special category, different in origin from gross rearrangements. If gene mutations are only special cases of minute rearrangements, then the frequency of minutes, like gene mutations and unlike gross structural changes, should vary linearly with the dose of radiation. A linear relation has been found (Muller, 1940) but, while it admits the possibility of an identity of origin and nature, the evidence does not constitute positive evidence that gene mutations are minute rearrangements.

One method of attack on the problem of whether minutes and gene mutations are different is to try other methods of inducing them, with the object of finding an agent which will discriminately induce one without the other. Ultraviolet light seems a possible agent, for although it induces breaks apparently no rearrangements result. The facts are discussed in the next section.

XIX. Genetical Effects of Ultraviolet Light

The induction of mutations by ultraviolet light has been studied in Drosophila (Altenburg, 1933, 1936; Demerec, Hollaender, Houlahan and Bishop, 1942; Muller and Mackenzie, 1934; Mackenzie and Muller, 1940; Mackenzie, 1941; Promptov, 1932), in Zea (Stadler, 1941; Stadler and Roman, 1943; Stadler and Sprague, 1936a, b, and c), in Antirrhinum (Stubbe and Noethling, 1936), in Sphaerocarpus (Knapp, 1938; Knapp and Schreiber, 1939), and in some fungi (Emmons and Hollaender, 1939a and b; Hollaender and Emmons, 1941; Hollaender *et al*, 1945; Lindegren and Lindegren, 1941). The low penetrating power of ultraviolet constitutes a technical difficulty in experiments with Drosophila. Some experiments have been made by irradiating fertilised eggs in the polar cap stage (Altenburg, 1934, 1936). More usually adult males have been used, the flies being held with their abdomena compressed between quartz plates and their undersides irradiated (Mackenzie and Muller, 1940; Demerec, Hollaender, Houlahan and Bishop, 1942). By this technique the testes are brought nearer to the surface, and a sufficient intensity of ultraviolet light reaches the sperm to induce a few per cent of mutations with the largest doses that do not inactivate the flies. However, absorption by the intervening tissue is large and variable, though it may be reduced by using a yellow stock which has less melanin in its body wall than has wild type. It is impossible to determine in Drosophila whether the yield

is strictly proportional to the dose or to compare the efficiencies of radiations of different wavelengths. It has been reported (Sell-Beleites and Catsch, 1942) that the yield of mutations induced by ultraviolet falls sharply at higher doses (fig. 22). The lower efficiency of higher doses in the case of maize is traced by Stadler and Uber (1942) to the eccentricity in the pollen grain of the sperm nucleus and the consequent selective effect on differently oriented pollen grains.

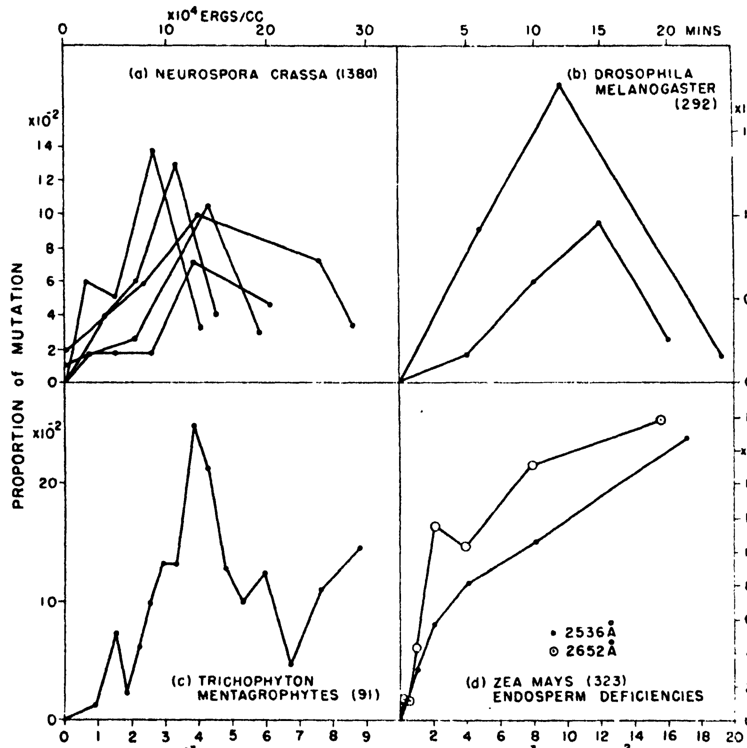


FIG. 22

Relation of ultraviolet induced mutation rate to the dosage of ultraviolet light.

In practice the greatest yields are obtained with a long wavelength, 3130 A, since the flies will tolerate greater doses of this wavelength than of shorter wavelengths, and absorption by the body wall is less so that relatively more of the incident light reaches the testes. However, it is probable that shorter wavelengths induce mutations more efficiently per unit of energy absorbed in the sperm. While ultraviolet will induce recessive lethal and visible mutations, gross structural changes are very rare in comparison with sex-linked lethals. Isolated instances of chromo-

some interchanges in ultraviolet irradiated material have been reported in Drosophila (Muller, 1941; Demerec *et al*, 1942), but their frequency does not seem to bear any relation to the dose received. Indeed Swanson (1942) finds the frequency of exchanges in Tradescantia pollen tubes to be independent of the ultra violet dose.

Compared with X-ray induced sex-linked lethals, it therefore appears that in Drosophila the category of type C lethals is rare in, or absent from, the lethals induced by ultraviolet light. Some ultraviolet induced lethals are said (Slizynski, 1942) to involve minute deficiencies (type B lethals) detectable in salivary chromosomes. In 21 X-chromosomes carrying induced lethals, 5 deficiencies were found with one band deficient, 3 with two bands deficient, and 1 with 16 bands deficient. This proportion is not different from that of type B lethals found amongst X-ray induced lethals. However, the matter is in doubt, since in other experiments (Mackenzie and Muller, 1940; Muller, 1941) no deletions of specific loci were found by genetic tests.

The low penetration of ultraviolet light is a less serious handicap when small objects, such as fungal spores and pollen grains, are used. In fungi, the yield of mutations is linearly proportional to the dose at low doses, but at high doses the proportion of the viable spores that carry mutants fails to rise any further and may diminish (fig. 22). This has been reported for *Trichophyton mentagrophytes* (Emmons and Hollaender, 1939a and b; Hollaender and Emmons, 1941) and for *Neurospora crassa* (Demerec, Kaufmann, Fano, Sutton, Sansome, 1942; Sansome *et al*, 1945) as well as for Drosophila (Sell-Beleites and Catsch, 1942). This peculiar and unexpected result appears at doses at which the fraction of irradiated fungal spores which survive is less than 1% and it suggests that the mutant spores have a lower viability than those which have not mutated. Amongst bacteria, radiation resistant mutants have been found, *e.g.* in *Escherichia coli* (Witkin, 1946). The observed relation between chromosome breakage and mutation (Sections XVI and XVIII) and the observed effect of a dose of ultraviolet in diminishing the breakability of chromosomes by X-rays (Swanson, 1944; Kaufmann, Hollaender and Gay, 1946) suggests that the greater resistance to mutation amongst the surviving spores (or sperm in Drosophila) may be caused by the earlier portion of the irradiation dose itself.

Stadler and his co-workers have described extensive experiments showing the genetic effects of ultraviolet irradiation of maize pollen and the differences between the effects of X-rays and ultraviolet light. The mature pollen is spread in a single layer, irradiated from above and then used to pollinate suitable ears. At the time of irradiation, the pollen contains two sperm nuclei, the generative nucleus having divided already.

Consequently the endosperm and embryo (and the plant grown from it) will show genetic effects produced independently of one another, so that progeny tests of effects detected in the endosperm are not possible.

In much of Stadler's work endosperm deficiencies were detected by irradiating pollen carrying the dominant allelomorphs of a number of genes affecting endosperm characters and then pollinating plants homozygous for the recessive allelomorphs of these genes. Some of the resultant grains showed the phenotypes of various of the recessives. This loss of effect of the dominant could be due either to mutation to a recessive allelomorph, or to loss (deletion) of the region of the chromosome containing the particular locus. In most cases deletion was responsible for the change, for when a chromosome carried two marked loci, these were very frequently lost together. The greater frequency of deficiencies observed is not because deficiencies are more frequent than random gene mutations, but because one deficiency involves many genes. Hence any particular locus is more likely to be affected by a deficiency than it is to undergo gene mutation.

Deficiencies have also been detected in plants, including maize, through the circumstance that, unless they are very small, they are lethal to pollen. Half of the pollen produced by a plant which is heterozygous for a deficiency is defective, and this can be recognized easily by inspection of the pollen. The deficiencies can usually be detected at the pachytene stage of meiosis in heterozygotes and with ultraviolet irradiation deficiencies of a large part of a chromosome arm are sometimes found (Rick, 1940; Singleton and Clark, 1940).

There are several interesting differences between the effects of X-rays and of ultraviolet light. The experimental data do not at present permit comparison of the absolute yields per unit dose of the two radiations, but marked qualitative differences and differences in the relative frequencies of different types of genetic effect occur.

The number of visible gene mutations relative to chromosome deficiencies in the F_1 plants is higher with ultraviolet light than with X-rays. This is shown by the ratio of total visible mutations scored in the F_2 generations to the total deficiencies detected in the F_1 plants by the segregation of defective pollen. It is also shown for a particular gene, *A*, by the fact that among 200 losses of A induced by X-rays, all involved at least some loss of viability and were probably deficiencies, none being simple mutation to a recessive allelomorph. Among a smaller number of losses caused by ultraviolet light, four were shown by their normal viability to be true mutations to a recessive allelomorph (Stadler, 1941; Stadler and Roman, 1943). Among spontaneous mutations at a number of loci, none showed evidence of loss of viability such as would suggest deficiency.

In most cases an endosperm deficiency produced by ultraviolet irradiation of the pollen affects only about half the endosperm. This suggests that the chromosomes in the sperm nuclei are already divided into two chromatids at the time of irradiation and that the ultraviolet light breaks only one of them. In experiments in which deficiencies for *A*, *Pr* and *Su* were recorded (Stadler and Uber, 1942), 80% of all deficiencies were fractional. The 20% of complete deficiencies could represent cases, where, by chance, both sister chromatids had been separately broken. That such chance coincidences may be frequent is suggested by the fact that in the same experiments 13% of the kernels were simultaneously deficient for two or more of the genes *A*, *Pr* and *Su*. With X-rays, however, the majority of endosperm deficiencies produced by irradiating the pollen are complete, suggesting that both chromatids are usually broken. The difference is accounted for on the facts that the ionizations of X-rays are localised on the paths of the ionizing particles while the quantum absorptions of ultraviolet light are strictly localised at discrete points. The chromatids of a chromosome being close together, an ionising particle which passes through one will usually pass through both.

Deficiencies for particular loci may be detected both in the endosperm and the F_1 plant. With X-rays they occur in the two kinds of tissue with equal frequency, as is to be expected since there is no reason to anticipate a difference in sensitivity between the two apparently identical and interchangeable sperm nuclei, one of which fertilizes the egg and the other the polar fusion nucleus. With ultraviolet light, however, there is a considerable difference, the deficiencies being much more frequent in the endosperm than in the F_1 plants. The difference could be explained as due to restitution of an ultraviolet induced break being less probable in the endosperm than in the embryo, possibly through the regular occurrence of sister-union at broken ends which occurs in endosperm and gametophyte but usually not in embryo tissues. Alternatively it may be that where a fractional deficiency would be expected in the zygote, some kind of elimination of the deficient portion occurs. In any case only one of the two cells resulting from the first division of the zygote takes any part in the formation of the embryo; consequently the number of deficiencies recovered in the F_1 plant could not exceed the total of the complete deficiencies together with half the fractional deficiencies.

Following X-ray treatment of pollen, deficiencies and interchanges are found with comparable frequency in F_1 plants. After ultraviolet light treatment of pollen, interchanges are rare and very much less frequent than deficiencies. Such interchanges as have been found are incomplete, only one of the two possible unions occurring; consequently an acentric fragment is lost. Since the ultraviolet induced deficiencies recovered in

the embryo are only a small fraction of those primarily produced, it is clear that ultraviolet light is able to produce breaks but that these have much less chance of combining to form interchanges than when the breaks are produced by X-rays. The cause of the difference is obscure, but the phenomenon seems to be general, as it occurs also in Drosophila and Tradescantia.

Deficiencies induced by ultraviolet appear to be exclusively terminal (Singleton, 1939; Singleton and Clark, 1940) while those induced by X-rays are mainly interstitial and dependent upon exchange between two breaks.

The relative efficiency of different wavelengths of ultraviolet light in inducing genetical changes have been compared (Stadler and Uber, 1942; Knapp *et al*, 1939; Hollaender and Emmons, 1941) in several organisms (fig. 23). In each case there is a rather close similarity between the

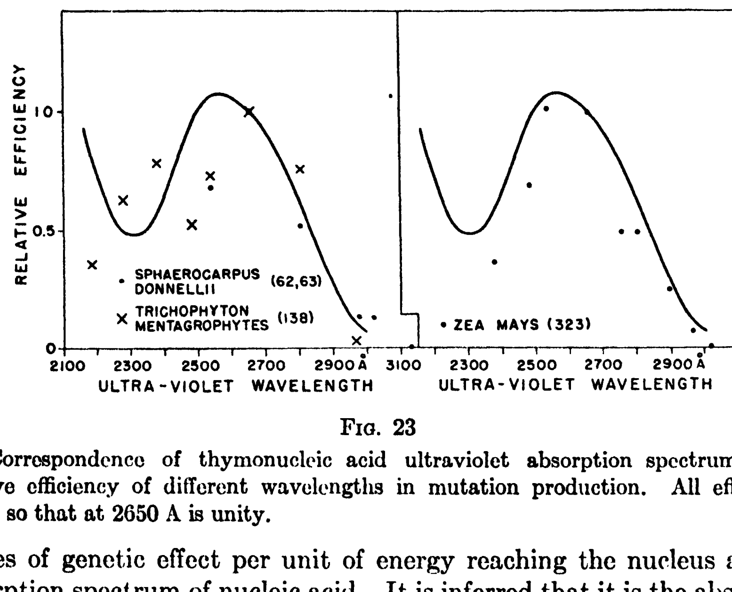


FIG. 23

Correspondence of thymonucleic acid ultraviolet absorption spectrum to the relative efficiency of different wavelengths in mutation production. All efficiencies scaled so that at 2650 A is unity.

curves of genetic effect per unit of energy reaching the nucleus and the absorption spectrum of nucleic acid. It is inferred that it is the absorption of ultraviolet light by the nucleoprotein of the chromosome that leads to the genetic effect, the nucleic acid being the active agent in the process.

XX. REFERENCES

1. Alberti, W. and Politzer, G., *Arch. mikr. Anat.* **100,** 83–109 (1923).
2. *Arch. mikr. Anat.* **103,** 284–307 (1924).
3. Alikhanian, S. I., *Zool. Zh. U.S.S.R.* **16,** 247–279 (1937).
4. Altenburg, E., *Amer. Nat.* **62,** 540–545 (1928).
5. *Science* **78,** 587 (1933).
6. *Amer. Nat.* **68,** 491–507 (1934).
7. *Biol. Zh.* **5,** 27–34 (1936).

8. Anderson, E. G., *Pap. Mich. Acad. Sci.* **4**, 523–525 (1924).
9. *Genetics* **16**, 386–396 (1931).
10. Anderson, E. G., and Randolph, L. F., *Genetics* **30**, 518–526 (1945).
11. Auerbach, C. and Robson, J. M., *Nature Lond.* **154**, 81 (1944).
12. *Nature* **157**, 302 (1946).
13. Babcock, E. B. and Collins, J. L., *Proc. nat. Acad. Sci.* **15**, 623–628 (1929).
14. Bauer, E., *Z. i. A. V.* **60**, 467–473 (1932).
15. Bauer, H., *Chromosoma* **1**, 343–390 (1939a).
16. *Naturwissenschaften* **27**, 821–822 (1939b).
17. *Proc. 7th Intern. Congr., Genet.* 58 (1939c).
18. Bauer, H., and Weschenfelder, R., *Naturwissenschaften* **26**, 820 (1938).
19. Bauer, H., Demerec, M. and Kaufmann, B. P., *Genetics* **23**, 610–630 (1938).
20. Beadle, G. W., and Tatum, E. L., *Amer. J. Bot.* **32**, 678–686 (1945).
21. Beleites, I., *Fundam. Radiol.* **5**, 142–152 (1939).
22. Belgovsky, M. L., *Genetica* **19**, 370–386 (1937).
23. *Bull. Inst. Genet. U.S.S.R.* **11**, 93–124 (1937).
24. *Bull. Acad. Sci., U.S.S.R. Ser. Biol.* 159–170 (1939).
24a. Belgovsky, M. L., and Muller, H. J., *Genetics* **23**, 139–140 (1937).
25. Bishop, D. W., *Genetics* **27**, 132 (1942).
26. Blanc, R., and Braun, W., *Univ. Calif. Publ. Zool.* **49**, 31–50 (1942).
27. Born, H. J., and Zimmer, K. G., *Z.i.A.V.* **78**, 246–250 (1940).
28. Bozeman, M. L., *Genetics* **28**, 71 (1943).
29. Brumfield, R. T., *Proc. nat. Acad. Sci.* **29**, 190–193 (1943).
30. Buchholz, J. T., and Blakeslee, A. F., *J. Hered.* **21**, 119–129 (1930).
31. Buchmann, W., and Hoth, I., *Z.i.A.V.* **57**, 355–363 (1937).
32. Buchmann, W., and Sydow, G., *Z.i.A.V.* **60**, 137–142 (1940).
33. Buchmann, W., and Zimmer, K. G., *Z.i.A.V.* **78**, 148–154 (1940).
34. *Z.i.A.V.* **79**, 192–198 (1941).
35. Carlson, J. G., *Genetics* **23**, 596–610 (1938a).
36. *Proc. nat. Acad. Sci.* **24**, 500–507 (1938b).
37. *Proc. nat. Acad. Sci.* **27**, 42–47 (1941a).
38. *Cold Spr. Harb. Symp. Quant. Biol.* **9**, 104–112 (1941b).
39. Cartledge, J. L., and Blakeslee, A. F., *Proc. nat. Acad. Sci.* **20**, 103–110 (1934).
40. Catcheside, D. G., *J. Genet.* **36**, 307–320 (1938a).
41. *J. Genet.* **36**, 321–328 (1938b).
42. *Proc. 7th Intern. Congr. Genet.*, 86 (1939).
43. *Biol. Rev.* **20**, 14–28 (1945).
44. *J. Genet.* **48**, 31–42, 99–110 (1947).
45. Catcheside, D. G., and Lea, D. E., *J. Genet.* **45**, 186–196 (1943).
46. *J. Genet.* **47**, 1–9 (1945a).
47. *J. Genet.* **47**, 25–40 (1945b).
48. Catcheside, D. G., Lea, D. E., and Thoday, J. M., *J. Genet.* **47**, 113–136 (1945a)
49. *J. Genet.* **47**, 137–149 (1945b).
50. Christie, A. L. M., *J. Genet.* **39**, 47–60 (1939).
51. Darlington, C. D., *Nature Lond.* **149**, 66 (1942).
52. Darlington, C. D., and La Cour, L. F., *J. Genet.* **46**, 180–267 (1945).
53. Darlington, C. D.. and Upcott, M. B., *J. Genet.* **41**, 297–338 (1941).
54. Delbrück, M., *Amer. Nat.* **74**, 350–362 (1940).
55. Demerec, M., *Proc. nat. Acad. Sci.* **19**, 1015–1020 (1933).
56. *Proc. nat. Acad. Sci.* **20**, 28–29 (1934a).

Demerec, M. (*continued*)
57. *Cold Spr. Harb. Symp. Quant. Biol.* **2,** 110 (1934b).
58. *Proc. nat. Acad. Sci.* **22,** 350–354 (1936).
59. *Cytologia, Fujii Jub Vol., Tokyo* 1125–1132 (1937).
60. *Radiology* **30,** 212–220 (1938).
61. *Science* **89,** 401 (1939a).
62. *Proc. 7th Intern. Congr. Genet.,* 99–103 (1939b).
63. *Genetics* **25,** 115–116 (1940a).
64. *Genetics* **25,** 618–622 (1940b).
65. *Proc. nat. Acad. Sci.* **32,** 36–46 (1946).
65a. *Science* **105,** 634 (1947).
66. Demerec, M., and Fano, U., *Proc. nat. Acad. Sci.* **27,** 24–31 (1941).
67. *Genetics* **29,** 348–360 (1944).
68. Demerec, M., Hollaender, A., Houlahan, M. B., and Bishop, M., *Genetics* **27,** 139–140 (1942).
68a. Demerec, M., and Kaufmann, B. P., *Yearb. Carneg. Instn.* **36,** 44–51 (1937).
69. Demerec, M., Kaufmann, B. P., Fano, U., Sutton, E., and Sansome, E. R., *Yearb. Carneg. Instn.* **41,** 190–199 (1942).
70. Demerec, M., Kaufmann, B. P., and Hoover, M. E., *Yearb. Carneg. Instn.* **37,** 40–47 (1938).
71. Demerec, M., Kaufmann, B. P., and Sutton, E., *Yearb. Carneg. Instn.* **38,** 185–191 (1939).
72. *Genetics* **27,** 140 (1942).
73. Demerec, M., Kaufmann, B. P., Sutton, E., and Hinton, O. T., *Yearb. Carneg. Instn.* **39,** 211–217 (1940).
74. Demerec, M., Kaufmann, B. P., Sutton, E., and Fano, U., *Yearb. Carneg. Instn.* **40,** 225–234 (1941).
75. Demerec, M., and Sutton, E., *Proc. nat. Acad. Sci.* **26,** 532–536 (1940).
76. Dempster, E. R., *Amer. Nat.* **75,** 184–187 (1940).
77. *Proc. nat. Acad. Sci.* **27,** 249–250 (1941).
78. Dobzhansky, Th., *Biol. Rev.* **11,** 364–384 (1936).
79. Döring, H. H., and Stubbe, H., *Z.i.A.V.* **75,** 352–357 (1938).
80. Dotterweich, H., *Zool. Anz. Suppl.* (*Verhandl. deutsch zool. Ges.* **41**) **12,** 244–253 (1939).
81. *Z.i.A.V.* **78,** 261–272 (1940).
82. Dotterweich, H., and Schmidtke, L., *Z.i.A.V.* **79,** 220–231 (1941a).
83. *Z.i.A.V.* **79,** 232–239 (1941b).
84. Dubinin, N. P., and Khvostova, V., *Biol. Zh.* **4,** 935–975 (1935).
85. Dubinin, N. P., and Sidorov, B., *Amer. Nat.* **68,** 377–381 (1934).
86. *Biol. Zh.* **4,** 555–568 (1935).
87. Dubinin, N. P., Sokolov, N. N., and Tiniakov, G. G., *Biol. Zh.* **4,** 707–720 (1935).
88. Eberhardt, K., *Chromosoma* **1,** 317–335 (1939).
88a. Efroimson, W. P., *Biol. Zbl.* **51,** 491–506 (1931).
89. Emerson, S. H., *Proc. nat. Acad. Sci.* **30,** 179–183 (1944).
90. Emmons, C. W., and Hollaender, A., *Genetics* **24,** 70–71 (1939a).
91. *Amer. J. Bot.* **24,** 467–475 (1939b).
92. Epsteins, F., *Genetica* **21,** 225–242 (1939).
93. Fabergé, A. C., *J. Genet.* **39,** 229–248 (1940a).
94. *J. Genet.* **40,** 379–384 (1940b).
95. Fano, U., *Cold Spr. Harb. Symp. Quant. Biol.* **9,** 113–120 (1941).

Fano, U. (*continued*)
96. *Quart. Rev. Biol.* **17,** 244–252 (1942).
97. *Proc. nat. Acad. Sci.* **29,** 12–18 (1943a).
98. *Genetics* **28,** 74–75 (1943b).
99. *Genetics* **29,** 361–369 (1944).
100. Fano, U., and Demerec, M., *Genetics* **26,** 251 (1941).
101. Fano, U., and Marinelli, L. D., *Proc. nat. Acad. Sci.* **29,** 59–66 (1943).
102. Friesen, H., *Z.i.A.V.* **71,** 501–526 (1936).
103. Fricke, H., and Demerec, M., *Proc. nat. Acad. Sci.* **23,** 320–327 (1937).
104. Fryer, H. C., and Gowen, J. W., *Genetics* **27,** 212–227 (1942).
105. Gelin, O. E. V., *Hereditas* **27,** 209–219 (1941).
106. Genter, C. F., and Brown, H. M., *J. Hered.* **32,** 39–44 (1941).
107. Gershenson, S., *C. R. Acad. Sci. U.S.S.R.* **25,** 236–238 (1939).
108. Giles, N. H., *Proc. nat. Acad. Sci.* **26,** 567–575 (1940).
109. *Genetics* **28,** 398–418 (1943).
110. Giles, N. H., and Nebel, B. R., *Genetics* **27,** 142–143 (1942).
111. Glass, H. B., *Amer. Nat.* **68,** 107–114 (1934).
112. *Genetics* **25,** 117 (1940a).
113. *Genetics* **25,** 118 (1940b).
114. Goodspeed, T. H., *Univ. Calif. Publ. Bot.* **11,** 285–298 (1930).
115. Goodspeed, T. H., and Avery, P., *Cytologia* **1,** 308–327 (1930).
116. Gowen, J. W., *Cold Spr. Harb. Symp. Quant. Biol.* **9,** 187–193 (1941).
117. Gowen, J. W., and Gay, E. H., *Genetics* **18,** 1–31 (1933).
118. Gray, L. H., *Proc. Camb. phil. Soc.* **40,** 72–102 (1943).
119. Grossman, E. F., and Smith, T., *Amer. Nat.* **67,** 429–436 (1933).
120. Gustafsson, A., *Hereditas* **27,** 225–242 (1941).
121. Hadorn, E., and Niggli, H., *Nature, Lond.* **157,** 162–163 (1946).
122. Hanson, F. B., *Amer. Nat.* **62,** 352–362 (1928).
123. *Physiol. Rev.* **13,** 466–496 (1933).
124. Hanson, F. B., and Heys, F., *Amer. Nat.* **63,** 201–213 (1929a).
125. *Amer. Nat.* **63,** 511–516 (1929b).
126. *Science* **71,** 43–44 (1930).
127. *Amer. Nat.* **66,** 335–345 (1932).
128. *Amer. Nat.* **67,** 127–135 (1933a).
129. *Amer. Nat.* **67,** 419–428 (1933b).
130. *Amer. Nat.* **67,** 567–571 (1933c).
131. *Amer. Nat.* **68,** 97–106 (1934).
132. Hanson, F. B., Heys, F., and Stanton, E., *Amer. Nat.* **65,** 134–143 (1931).
133. Hanson, F. B., and Winkelman, E., *J. Hered.* **20,** 277–286 (1929).
134. Harris, B. B., *J. Hered.* **20,** 299–302 (1929).
135. Helfer, R. G., *Proc. nat. Acad. Sci.* **26,** 3–7 (1940).
136. *Genetics* **26,** 1–22 (1941).
137. Hersh, A. H., Karrer, E., and Loomis, A. L., *Amer. Nat.* **64,** 552–559 (1930).
137a. Herskowitz, I. H., *Amer. Nat.* **80,** 588–592 (1946).
137b. Hertwig, P., *Proc. 7th Intern. Congr. Genet.*, 145–146 (1939).
138. Hollaender, A., and Emmons, C. W., *Cold Spr. Harb. Symp. Quant. Biol.* **9,** 179–186 (1941).
138a. Hollaender, A., Sansome, E. R., Zimmer, E., and Demerec, M., *Amer. J. Bot.* **32,** 226–235 (1945).
139. Hoover, M. E., *Z.i.A.V.* **74,** 420–434 (1938).

140. Horlacher, W. R., *Science* **72,** 96–97 (1930).
141. Johnson, O., and Winchester, A. M., *Amer. Nat.* **68,** 351–358 (1934).
142. Jollos, V., *Genetics* **24,** 113–130 (1939).
143. Kanellis, A., *Z.i.A.V.* **81,** 191–195 (1943).
144. Kaplan, R., *Z.i.A.V.* **77,** 568–579 (1939).
145. Kaufmann, B. P., *Proc. nat. Acad. Sci.* **25,** 571–577 (1939a).
146. *Rec. Genet. Soc. Amer.* **7,** 101 (1939b).
147. *Proc. nat. Acad. Sci.* **27,** 18–24 (1941a).
148. *Cold Spr. Harb. Symp. Quant. Biol.* **9,** 82–92 (1941b).
149. *Genetics* **26,** 157 (1941c).
150. *Proc. nat. Acad. Sci.* **29,** 8–12 (1943a).
151. *Rec. Genet. Soc. Amer.* **12,** 50 (1943b).
152. Kaufmann, B. P., and Bate, R. C., *Proc. nat. Acad. Sci.* **24,** 368–371 (1938).
153. Kaufmann, B. P., and Demerec, M., *Proc. nat. Acad. Sci.* **23,** 484–488 (1937).
154. Kaufmann, B. P., and Hollaender, A., *Genetics* **31,** 368–376 (1946).
155. Kaufmann, B. P., Hollaender, A., and Gay, H., *Genetics* **31,** 349–367 (1946).
156. Kerkis, J., *C. R. Acad. Sci. U.S.S.R.* **1,** 55–61 (1935a).
157. *Bull. Inst. Genetics* **10,** 223–226 (1935b).
158. Khvostova, V. V., and Gavrilova, A. A., *Biol. Zh.* **4,** 905–916 (1935).
159. *Biol. Zh.* **7,** 381–390 (1938).
160. Knapp, E., *Z.i.A.V.* **70,** 309–349 (1935).
161. *Z.i.A.V.* **74,** 54–69 (1938).
162. Knapp, E., Reuss, A., Risse, O., and Schreiber, H., *Naturwissenschaften* **27,** 304 (1939).
163. Knapp, E., and Schreiber, H., *Proc. 7th Intern. Congr. Genet.,* 175 (1939).
164. Koller, P. C., *Proc. Roy. Soc. Edin. B* **61,** 398–429 (1943).
165. Koller, P. C., and Ahmed, I. A. R. S., *J. Genet.* **44,** 53–72 (1942).
166. Kondakova, A. A., *Biol. Zh.* **4,** 721–726 (1935).
167. Kossikoff, K. V., *Bull. Inst. Genetics* **10,** 189–197 (1935a).
168. *Genetics* **20,** 363–365 (1935b).
169. Kotval, J. P., and Gray, L. H., *J. Genet.* **48,** 135–154 (1947).
170. Lea, D. E., *J. Genet.* **39,** 181–188 (1940a).
171. *Nature* **146,** 137 (1940b).
172. *Actions of Radiations on Living Cells.* Cambridge Univ. Press, Cambridge (1945).
173. Lea, D. E., and Catcheside, D. G., *J. Genet.* **44,** 216–245 (1942).
174. *J. Genet.* **47,** 10–24 (1945a).
175. *J. Genet.* **47,** 41–50 (1945b).
176. Lea, D. E., and Salaman, M. H., *Brit. J. Exp. Path.* **23,** 27–37 (1942).
177. Lea, D. E., and Smith, K. M., *Parasitology* **34,** 227–237 (1942).
178. Lindegren, C. C., and Lindegren, G., *J. Hered.* **32,** 435–440 (1941).
179. Lobasov, M. E., *Biol. Zh.* **7,** 581–594 (1938).
180. Lobasov, M. E., and Smirnov, F., *C. R. Acad. Sci. U.S.S.R.* **2,** 307–311 (1934a).
181. *C. R. Acad. Sci. U.S.S.R.* **3,** 174–178 (1934b).
182. McClintock, B., *Res. Bull. Mo. agric. Exp. Sta.* **290,** 1–48 (1938a).
183. *Genetics* **23,** 315–376 (1938b).
184. *Proc. nat. Acad. Sci.* **25,** 405–416 (1939).
185. *Cold Spr. Harb. Symp. Quant. Biol.* **9,** 72–81 (1941a).
186. *Genetics* **26,** 234–282 (1941b).
187. *Genetics* **26,** 542–571 (1941c).
188. *Proc. nat. Acad. Sci.* **28,** 458–463 (1942a).

McClintock, B. (*continued*)
188a. *Yearb. Carneg. Instn.* **41,** 181–186 (1942b).
188b. *Genetics* **29,** 478–502 (1944a).
188c. *Yearb. Carneg. Instn.* **43,** 127–135 (1944b).
189. Mackenzie, K., *Proc. roy. Soc. Edinb.* B **61,** 67–77 (1941).
190. Mackenzie, K., and Muller, H. J., *Proc. roy. Soc.* B **129,** 494–517 (1940).
191. Mainz, F., *Z.i.A.V.* **78,** 238–245 (1940).
192. Magrzhikovskaya, K. W., *Biol. Zh.* **7,** 635–642 (1938).
193. Marinelli, L. D., Nebel, B. R., Giles, N. H., and Charles, D. R., *Amer. J. Bot.* **29,** 866–874 (1942).
194. Marquardt, H., *Z. Bot.* **32,** 401–482 (1938).
195. Marshak, A., *Proc. nat. Acad. Sci.* **23,** 362–369 (1937).
196. *Proc. Soc. exp. Biol., N. Y.* **38,** 705–713 (1938a).
197. *Proc. Soc. exp. Biol., N. Y.* **39,** 194–198 (1938b).
198. *Proc. Soc. exp. Biol., N. Y.* **41,** 176–180 (1939).
199. *Proc. nat. Acad. Sci.* **28,** 29–35 (1942a).
200. *Radiology* **39,** 621–626 (1942b).
201. Marshak, A., and Bradley, M., *Proc. nat. Acad. Sci.* **30,** 231–237 (1944).
202. Marshak, A., and Malloch, W. S., *Genetics* **27,** 576–583 (1942).
203. Mather, K., *Proc. roy. Soc.* B **124,** 97–106 (1937).
204. Mather, K., and Stone, L. H. A., *J. Genet.* **28,** 1–24 (1933).
205. Maxwell, J., *Biol. Bull.* **74,** 253–255 (1938).
206. Medvedev, N. N., *C. R. Acad. Sci. U.S.S.R.* **5,** 1–7 (1933).
207. *Bull. Inst. Genetics* **10,** 211–222 (1935a).
208. *C. R. Acad. Sci. U.S.S.R.* **4,** 283–285 (1935b).
209. Metz, C. W., and Bozeman, M. L., *Proc. nat. Acad. Sci.* **26,** 228–231 (1940).
210. Mickey, G. H., *Genetics* **23,** 160 (1938).
211. *Genetica* **21,** 386–407 (1939).
212. Millikan, C. R., *J. Australian Inst. Agric. Sci.* **6,** 203–205 (1940).
213. Mitchell, J. S., *Brit. J. exp. Path.* **23,** 285–313 (1942).
214. Moore, W. G., *Genetics* **29,** 209–222 (1934).
215. Muller, H. J., *Science* **66,** 84–87 (1927).
216. *Proc. nat. Acad. Sci.* **14,** 714–726 (1928).
217. *Proc. Intern. Congr. Plt. Sci.* **1,** 897 (1929).
218. *Amer. Nat.* **64,** 220–251 (1930a).
219. *J. Genet.* **22,** 299–334 (1930b).
220. *Amer. Nat.* **69,** 405–411 (1935a).
220a. *Genetica* **17,** 237–252 (1935b).
221. *Reun. int. Phys. Chim. Biol.* **8,** 477–494 (1938a).
222. *Collecting Net* **13,** 181–198 (1938b).
223. *Proc. 7th Intern. Congr. Genet.* 221–222 (1939).
224. *J. Genet.* **40,** 1–66 (1940).
224a. *The New Systematics.* 185–268. Ed. J. S. Huxley (1940). Clarendon Press, Oxford.
225. *Cold Spr. Harb. Symp. Quant. Biol.* **9,** 151–165 (1941).
226. Muller, H. J., and Altenburg, E., *Anat. Rec.* **41,** 100 (1928).
227. Muller, H. J., and Altenburg, E., *Genetics* **15,** 283–311 (1930).
228. Muller, H. J., and Mackenzie, K., *Nature* **143,** 83 (1939).
229. Muller, H. J., and Mott-Smith, L. M., *Proc. nat. Acad. Sci.* **16,** 277–285 (1930).

230. Muller, H. J., and Pontecorvo, G., *Genetics* **27**, 157–158 (1942).
231. Muller, H. J., and Prokofyeva, A. A., *C. R. Acad. Sci. U.S.S.R.* **4**, 74–83 (1934).
232. Muller, H. J., Prokofyeva, A. A., and Raffel, D., *Nature, Lond.* **135**, 253–255 (1935).
233. Muller, H. J., and Raffel, D., *Nature, Lond.* **135**, 253–255 (1935).
234. Müntzing, A., *Hereditas, Lund* **28**, 217–221 (1942).
235. Nagai, M. A., and Locher, G. L., *Genetics* **23**, 179–189 (1935).
236. Nebel, B. R., *Cytologia, Tokyo* **5**, 1–14 (1933).
237. *Amer. J. Bot.* **24**, 365–372 (1937).
238. Nebel, B. R., Wilson, G. B., and Marinelli, L., *Genetics* **27**, 158 (1942).
239. Neuhaus, M., *Biol. Zh.* **3**, 602–608 (1934).
240. Newcombe, H. B., *J. Genet.* **43**, 145–171 (1942a).
241. *J. Genet.* **43**, 237–248 (1942b).
242. Nichols, C., *Amer. J. Bot.* **29**, 755–759 (1942).
243. Nishina, Y., and Moriwaki, D., *Sci. Pap. Inst. phys. chem. Res. Tokyo* **36**, 419–425 (1939).
244. *Sci. Pap. Inst. phys. chem. Res. Tokyo* **38**, 371–376 (1941).
245. Noethling, W., and Stubbe, H., *Z.i.A.V.* **67**, 152–172 (1934).
246. *Verh.* **3** *Int. Kongr. Lichtforschung*, 238–246 (1936).
247. Oliver, C. P., *Z.i.A.V.* **61**, 447–488 (1932).
248. Pätau, K., and Timoféeff-Ressovsky, N. W., *Z.i.A.V.* **81**, 62–71 (1943).
249. *Z.i.A.V.* **81**, 181–190 (1943).
250. Patterson, J. T., *Biol. Bull.* **61**, 133–138 (1931).
251. *Amer. Nat.* **66**, 193–206 (1932).
252. Patterson, J. T., and Suche, M. L., *Genetics* **19**, 223–236 (1934).
253. Panshin, I. B., *C. R. Acad. Sci. U.S.S.R.* **4**, 85–88 (1935).
254. Papalashwili, G., *Biol. Zh.* **4**, 587–591 (1935).
255. Pekarek, J., *Planta* **4**, 299–357 (1927).
256. Peto, F. H., *Canad. J. Agric. Res.* **9**, 261–264 (1933).
256a. Pickhan, A., *Strahlentherapie* **52**, 369–388 (1935).
256b. Pickhan, A., Timoféeff-Ressovsky, N. W., and Zimmer, K. G., *Strahlentherapie* **56**, 488 (1936).
257. Plough, H. H., and Ives, P. T., *Proc. nat. Acad. Sci.* **20**, 268–273 (1934).
258. Ponomarev, V. P., *Biol. Zh.* **6**, 69–80 (1937).
259. *Biol. Zh.* **7**, 619–633 (1938).
260. Pontecorvo, G., *J. Genet.* **41**, 195–215 (1941).
261. *J. Genet.* **43**, 295–300 (1942).
262. Pontecorvo, G., and Muller, H. J., *Genetics* **26**, 165 (1941).
263. Prokofyeva-Belgovskaya, A. A., *C. R. Acad. Sci. U.S.S.R.* **22**, 270–273 (1939).
264. Promptov, A. N., *J. Genet.* **26**, 59–74 (1932).
265. Rajewsky, B. N., Krebs, A., and Ziekler, H., *Naturwissenschaften* **24**, 619 (1936).
266. Rajewsky, B. N., and Timoféeff-Ressovsky, N. W., *Z.i.A.V.* **77**, 488–500 (1939).
267. Ray-Chaudhuri, S. P., *Proc. roy. Soc. Edinb.* B **62**, 66–72 (1944).
268. Reynolds, J. P., *Proc. nat. Acad. Sci.* **27**, 204–208 (1941).
269. Rick, C. M., *Genetics* **25**, 466–482 (1940).
270. *Proc. nat. Acad. Sci.* **28**, 518–525 (1942).
271. *Genetics* **28**, 237–252 (1943).
272. Riley, H. P., *Cytologia, Tokyo* **7**, 131–142 (1936).
272a. Ryan, F. J., and Lederberg, J., *Proc. nat. Acad. Sci.* **32**, 163–173 (1946).
273. Sacharov, V. V., *Biol. Zh.* **4**, 107–111 (1936a).
274. *Genetica* **18**, 193–216 (1936b).

275. Sansome, E. R., Demerec, M., and Hollaender, A., *Amer. J. Bot.* **32**, 218–226 (1945).
276. Sax, H. J., and Sax, K., *J. Arnold Arbor.* **16**, 423–439 (1935).
277. Sax, K., *Genetics* **23**, 494–516 (1938).
278. *Proc. nat. Acad. Sci.* **25**, 225–233 (1939).
279. *Genetics* **25**, 41–68 (1940).
280. *Genetics* **26**, 418–425 (1941a).
281. *Cold Spr. Harb. Symp. Quant. Biol.* **9**, 93–101 (1941b).
282. *J. Gen. Physiol.* **25**, 533–537 (1942a).
283. *Proc. nat. Acad. Sci.* **28**, 229–233 (1942b).
284. *Proc. nat. Acad. Sci.* **29**, 18–21 (1943).
284a. *Genetics* **32**, 75–78 (1947).
285. Sax, K., and Brumfield, R. T., *Amer. J. Bot.* **30**, 564–570 (1943).
286. Sax, K., and Enzmann, E. V., *Proc. nat. Acad. Sci.* **25**, 397–405 (1939).
287. Sax, K., and Mather, K., *J. Genet.* **37**, 483–490 (1939).
288. Sax, K., and Swanson, C. P., *Amer. J. Bot.* **28**, 52–59 (1941).
238a. Schechtmann, J., *J. Exp. Biol. U.S.S.R.* **6**, 271–279 (1930).
289. Schmitt, F. O., and Oliver, C. P., *Amer. Nat.* **67**, 67–68 (1933).
290. Schultz, J., in Biological Effects of Radiation, 1209–1261 (Ed. 1936, B. M. Duggar) McGraw-Hill, New York.
291. Schütze, *Z.i.A.V.* **81**, 484–499 (1943).
292. Sell-Beleites, I., and Catsch, A., *Z.i.A.V.* **80**, 551–557 (1942).
293. Serebrovskaya, R. T., and Shapiro, N. J., *C. R. Acad. Sci. U.S.S.R.* **2**, 421–428 (1935).
293a. Serebrovsky, A. S., *Amer. Nat.* **63**, 374–378 (1929).
293b. Shapiro, N. J., and Neuhaus, M., *Biol. Zh.* **2**, 425–446 (1933).
294. Sidky, A. R., *Amer. Nat.* **74**, 475–480 (1940).
295. Sidorov, B. N., *Zh. Exp. Biol.* **7**, 349–351 (1931).
296. Singleton, W. R., *Genetics* **24**, 109 (1939).
297. Singleton, W. R., and Clark, F. J., *Genetics* **25**, 136 (1940).
298. Sizova, M. A., *C. R. Acad. Sci. U.S.S.R.* **2**, 197–198 (1936).
299. Slizynski, B. M., *Genetics* **23**, 283–290 (1938).
300. *Proc. Roy. Soc. Edinb.* B **61**, 297–315 (1942).
301. Smirnov, F. A., *Trav. Soc. Nat. Leningrad* **63**, 379–385 (1934).
302. Snell, G. D., *Genetics* **20**, 545 (1935).
303. *Proc. nat. Acad. Sci.* **25**, 11–14 (1939).
304. *Radiology* **36**, 189 (1941).
305. Sommermeyer, K., *Z.i.A.V.* **79**, 240–244 (1941).
306. Sonnenblick, B. P., *Proc. nat. Acad. Sci.* **26**, 373–381 (1940).
306a. Sparrow, A. H., *Proc. nat. Acad. Sci.* **30**, 147–155 (1944).
307. Stadler, L. J., *Science* **68**, 186–187 (1928a).
308. *Proc. nat. Acad. Sci.* **14**, 69 (1928b).
309. *Anat. Rec.* **41**, 381 (1928c).
310. *Proc. nat. Acad. Sci.* **16**, 714–720 (1930).
311. *J. Hered.* **21**, 3–19 (1930).
312. *Sci. Agric.* **11**, 557–572 (1931).
313. *Sci. Agric.* **11**, 645–661 (1931).
314. *Proc. 6th Intern. Congr. Genet.* **1**, 274–294 (1933).
315. *Proc. 7th Intern. Congr. Genet.* 269–276 (1939).
316. *Cold Spr. Harb. Symp. Quant. Biol.* **9**, 168–178 (1941).
316a. *Proc. nat. Acad. Sci.* **30**, 123–128 (1944).

317. Stadler, L. J., and Roman, H., *Genetics* **28**, 91 (1943).
318. Stadler, L. J., and Sprague, G. F., *Proc. nat. Acad. Sci.* **22**, 572–578 (1936a).
319. *Proc. nat. Acad. Sci.* **22**, 579–583 (1936b).
320. *Proc. nat. Acad. Sci.* **22**, 584–591 (1936c).
321. *Science* **85**, 57–58 (1937).
322. Stadler, L. J., and Uber, F. M., *Genetics* **23**, 171 (1938).
323. *Genetics* **27**, 84–118 (1942).
324. Steinberg, R. A., and Thom, C., *J. Hered.* **31**, 61–63 (1940).
325. Stern, C., and Heidenthal, G., *Proc. nat. Acad. Sci.* **30**, 197–205 (1944).
326. Stevens, W. L., *J. Genet.* **43**, 301–307 (1942).
327. Stubbe, H., *Z.i.A.V.* **56**, 1–38 (1930).
328. *Z.i.A.V.* **60**, 474–513 (1932).
329. *Z.i.A.V.* **64**, 181–204 (1933).
330. *Z.i.A.V.* **70**, 161–169 (1935).
331. *Biol. Zbl.* **60**, 113–129 (1940).
332. Stubbe, H., and Döring, H., *Z.i.A.V.* **75**, 341–351 (1938).
333. Stubbe, H., and Noethling, W., *Z.i.A.V.* **72**, 378–386 (1936).
333a. Sutton, E., *Genetics* **25**, 628–635 (1940).
334. Swanson, C. P., *Proc. nat. Acad. Sci.* **26**, 366–373 (1940).
335. *Genetics* **27**, 491–503 (1942).
336. *J. Gen. Physiol.* **26**, 485–494 (1943).
337. *Genetics* **29**, 61–68 (1944).
337a. Swanson, C. P., and Hollaender, A., *Proc. nat. Acad. Sci.* **32**, 295–302 (1946).
338. Tatum, E. L., *Proc. nat. Acad. Sci.* **31**, 215–219 (1945).
339. Thoday, J. M., *J. Genet.* **43**, 189–210 (1942).
339a. Thoday, J. M., and Read, J., *Nature* **160**, 608 (1947).
340. Timoféeff-Ressovsky, N. W. *Ergeb. med. Strahlenforsch.* **5**, 130–228 (1930).
341. *J. Hered.* **21**, 167–171 (1931).
342. *Arch. EntwMech. Org.* **124**, 654–665 (1931).
343. *Z.i.A.V.* **64**, 173–175 (1932).
344. *Z.i.A.V.* **65**, 278–292 (1933a).
345. *Z.i.A.V.* **66**, 165–179 (1933b).
346. *Strahlentherapie* **49**, 463–478 (1934a).
347. *Biol. Rev.* **9**, 411–457 (1934b).
348. *Strahlentherapie* **51**, 658–663 (1934c).
349. *Z.i.A.V.* **70**, 125–129 (1935).
350. *Biol. Zbl.* **57**, 309–315 (1937).
351. Experimentelle Mutationsforschung in der Vererbungslehre. Steinkopf. Dresden and Leipzig (1937).
352. *Proc. 7th Intern. Congr. Genet.*, 281–294 (1939a).
353. *Chromosoma* **1**, 310–316 (1939b).
354. *Biol. Zbl.* **60**, 267–275 (1940).
355. Timoféeff-Ressovsky, N. W., and Delbrück, M., *Z.i.A.V.* **71**, 322–334 (1936).
356. Timoféeff-Ressovsky, N.W., and Zimmer, K.G., *Strahlentherapie* **53**, 134–138 (1935a).
357. *Strahlentherapie* **54**, 265–278 (1935b).
358. *Naturwissenschaften* **26**, 108 (1938a).
359. *Naturwissenschaften* **27**, 362–365 (1938b).
360. *Biol. Zbl.* **59**, 358–362 (1939a).
361. *Strahlentherapie* **66**, 684 (1939b).
362. *Z.i.A.V.* **79**, 530–537 (1941).

363. Timoféeff-Ressovsky, N. W., Zimmer, K. G., and Delbrück, M., *Nachr. Ges. Wiss.*, Göttingen **1,** 163–241 (1935).
364. Ward, F. D., *Genetics* **20,** 230–249 (1935).
365. Weaver, E. A., *Thesis; Univ. of Missouri* 93 pp. (1942). (Abstract seen).
366. Weinstein, A., *Science* **67,** 376–377 (1928).
367. Whelden, R. M., *Mycologia* **32,** 630–643 (1940).
368. White, M. J. D., *Proc. roy. Soc.* B **119,** 61–84 (1935).
369. *Proc. roy. Soc.* B **124,** 183–196 (1937).
370. Whiting, A. R., *Biol. Bull.* **89,** 61–71 (1945).
371. Whiting, P. W., *Proc. Pa. Acad. Sci.* **11,** 50–52 (1937).
372. *Proc. Pa. Acad. Sci.* **12,** 74–76 (1938a).
373. *Genetics* **23,** 562–572 (1938b).
374. Whittinghill, M., *Genetics* **22,** 114–129 (1937).
375. Wilhelmy, E., Timoféeff-Ressovsky, N. W., and Zimmer, K. G., *Strahlentherapie* **57,** 521–531 (1936).
376. Witkin, E. M., *Proc. nat. Acad. Sci.* **32,** 59–68 (1946).
377. Wollman, E., Holweck, F., and Luria, S., *Nature, Lond.*, **145,** 935–936 (1940).
378. Zimmer, K. G., *Strahlentherapie* **51,** 179–184 (1934).
379. Zimmer, K. G., and Timoféeff-Ressovsky, N. W., *Strahlentherapie* **55,** 77–84 (1936).
380. *Strahlentherapie* **63,** 528–536 (1938).
381. Zimmer, K. G., and Timoféeff-Ressovsky, N. W., *Z.i.A.V.* **80,** 353–372 (1942).

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